WEIZMANN INSTITUTE OF SCIENCE

The metabolome of duckweed – MS based natural product identification and flux analysis

Uwe Heinig

Weizmann Institute of Science

13.07.2017

Overview

Part I – metabolic profiling of *Lemnaceae*

Part II – DLEMMA for metabolite identification

Part III – flux analysis using *Spirodela polyrhiza*

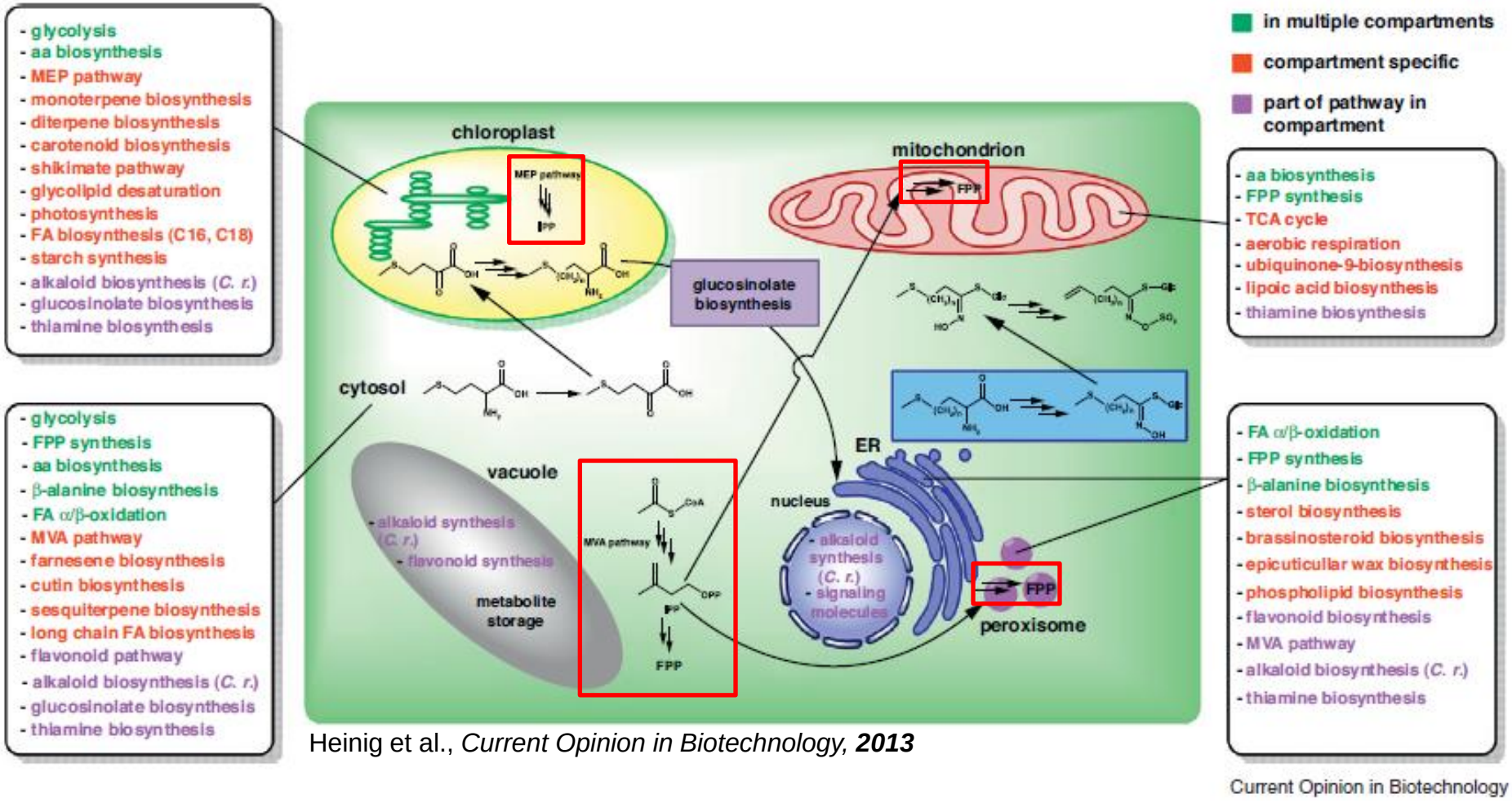
Overview

Part I – metabolic profiling of *Lemnaceae*

Part II – DLEMMA for metabolite identification

Part III – flux analysis using *Spirodela polyrhiza*

The plant metabolic network



Heinig et al., *Current Opinion in Biotechnology*, ***2013***

→ **Identification of metabolites (I) -> annotation of reactions/metabolic network (II)**

→ **pathways for flux studies (III)**

Analysis and identification of natural products – techniques

	MS	NMR
sensitivity	+++	+
Identification level	+	+++
Standards Spectral libraries	++	+
Costs/speed	+++	+

➢ ***Mass spectrometry (coupled to chromatography) in combination with computational tools method of choice***

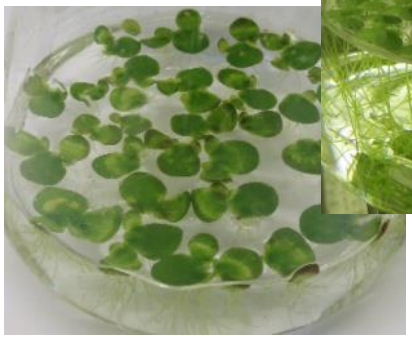
Lemna – what are Lemnacea?

→ free floating water plant of the duckweed family

→ reproduces mainly vegetatively

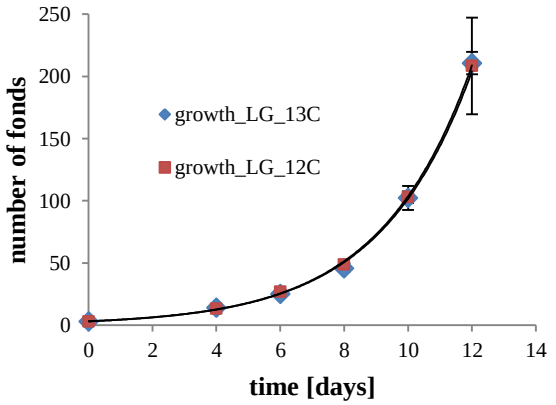
(two daughter fronds from one adult plant)

Lemna gibba

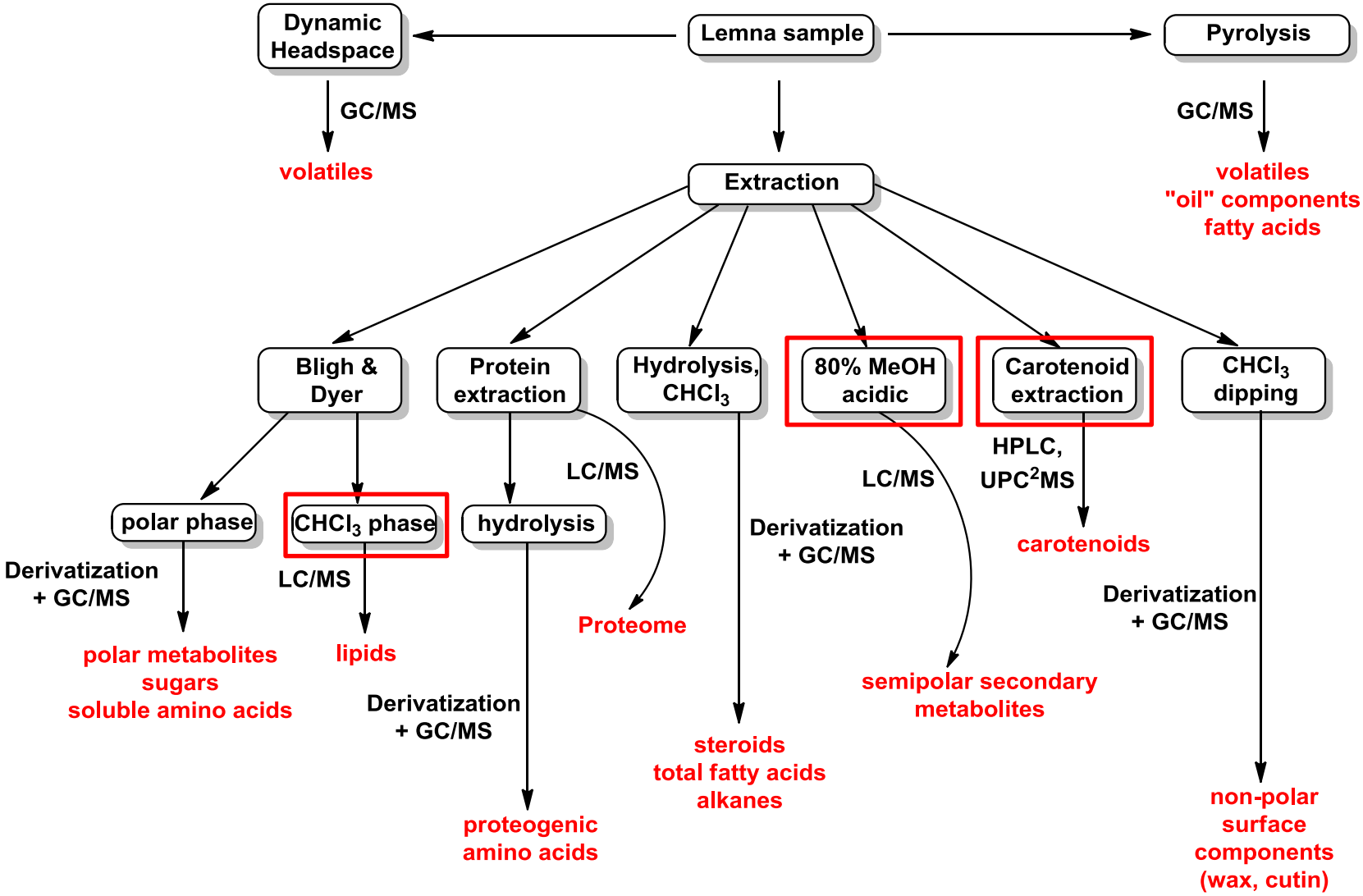


Spirodela polyrhiza

- → **High protein & starch content / low lignin**
- → **High vitamin levels (β-carotene)**
- → **Vegetative reproduction**
- → **Metabolite profiling**
- → **Metabolic model reconstruction**
- → **Metabolic flux analysis of "whole plants"**
- → **Metabolic engineering**



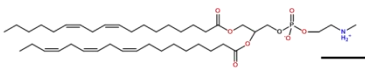
Lemna – profiling - overview



Lemna – profiling – results – 4 species

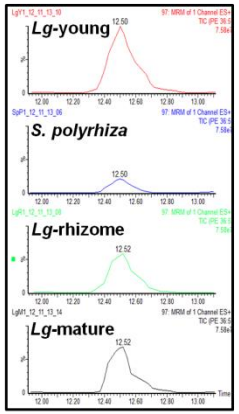
Lipid analysis

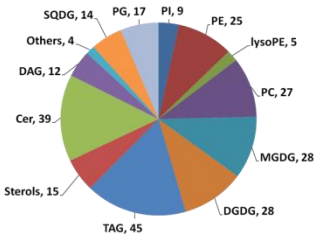
Chloroform extracts were subjected to LC-TQ-MS analysis – Example:



Phosphatidylethanolamine (36:5)

SRM-transition
m/z 738.51 --> 597.49

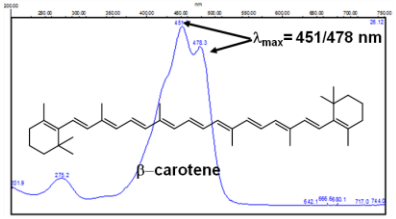


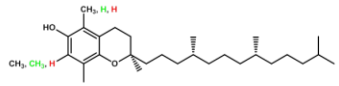


→ **Identification of 291 different lipid species, relative quantification, comparison between species & different tissues (*Lemna gibba*: mature & young plant + rhizome)**

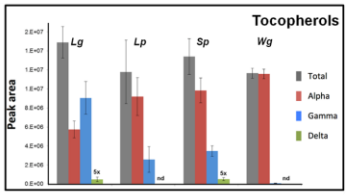
Isoprenoid analysis

Chloroform extracts were subjected HPLC- UV/Fluorescence analysis ; compounds were identified by comparison to authentic standards or absorption characteristics:



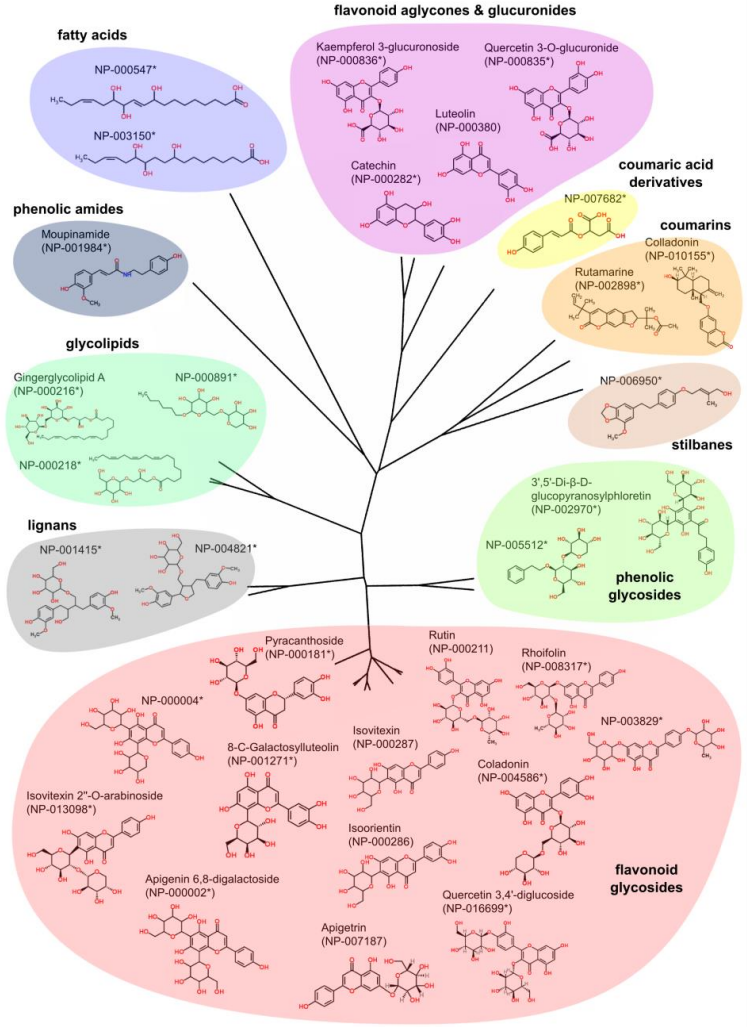


α, γ, δ - Tocopherol



→ **Identification of 3 tocopherols, 19 carotenoids, chlorophyll a & b and 6 unknown compounds**

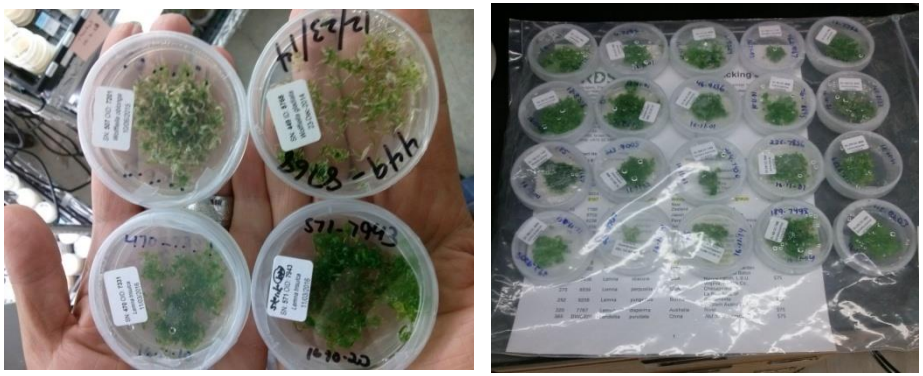
Semipolar secondary metabolites (WISmass)



WEIZMANN INSTITUTE OF SCIENCE

The AA-Lemna collection

- **33 duckweed species from RDSC Rutgers Duckweed Stock Cooperative**
- **all 5 genera of *Lemnaceae* family (Spirodela (6), Lemna (14), Landoltia (2), Wolffia (8) & Wolffiella (3))**
- **"covering the family"**
- **including sequenced strains of *Spirodela polyrhiza*, *Lemna gibba* & *Lemna minor***

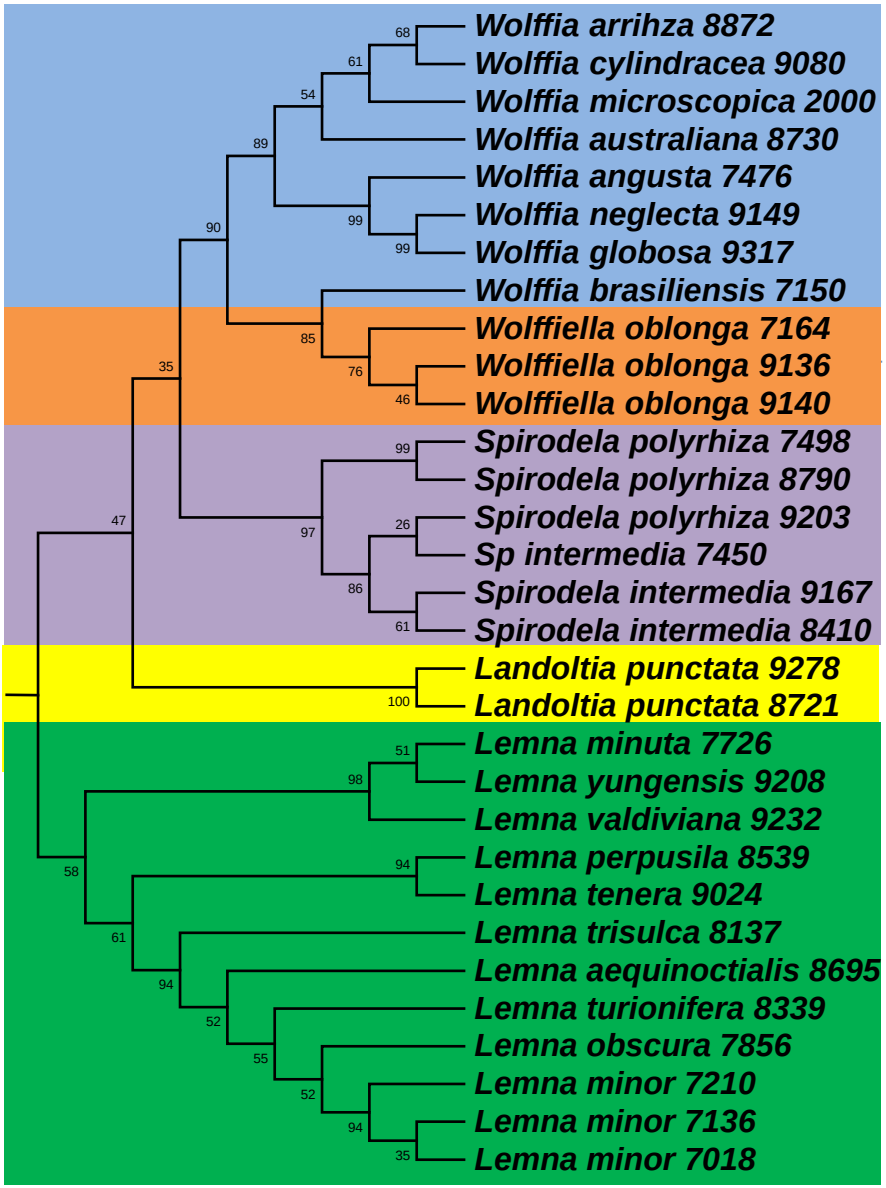


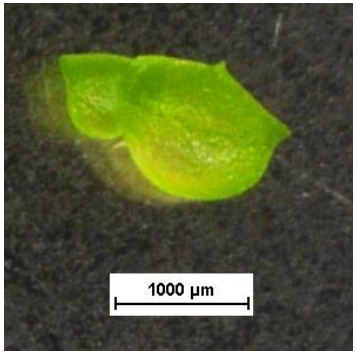
Various *Lemna* strains of the RDSC collection

WEIZMANN INSTITUTE OF SCIENCE

The AA-Lemna collection – geographical distribution

The AA-Lemna collection – diversity

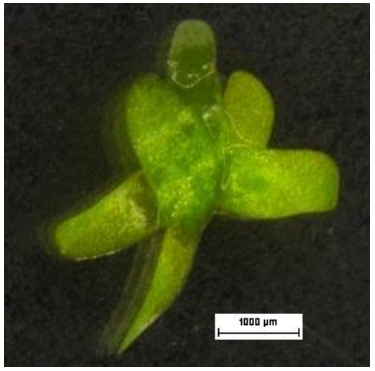




Wolffia sp.

Spirodela sp.

Lemna sp.

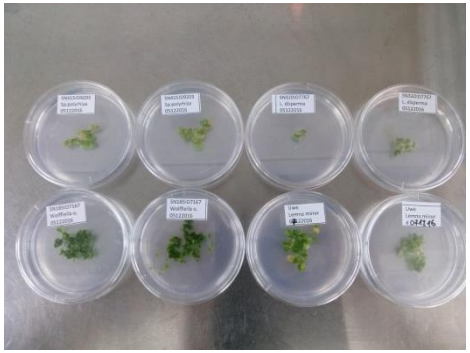


Wolffiella sp.

Landoltia sp.

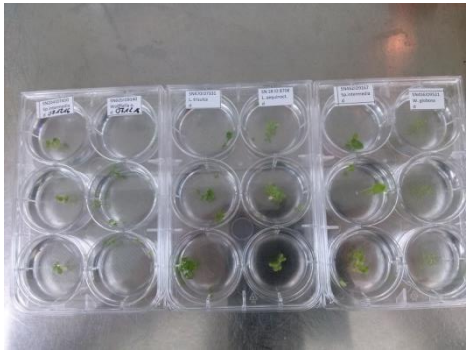
WEIZMANN INSTITUTE OF SCIENCE

The AA-Lemna collection – growth & LC-MS analysis



Transfer to 6-well plates, SP-medium

Cultivation for 12 d
Constant light, 3% CO_2

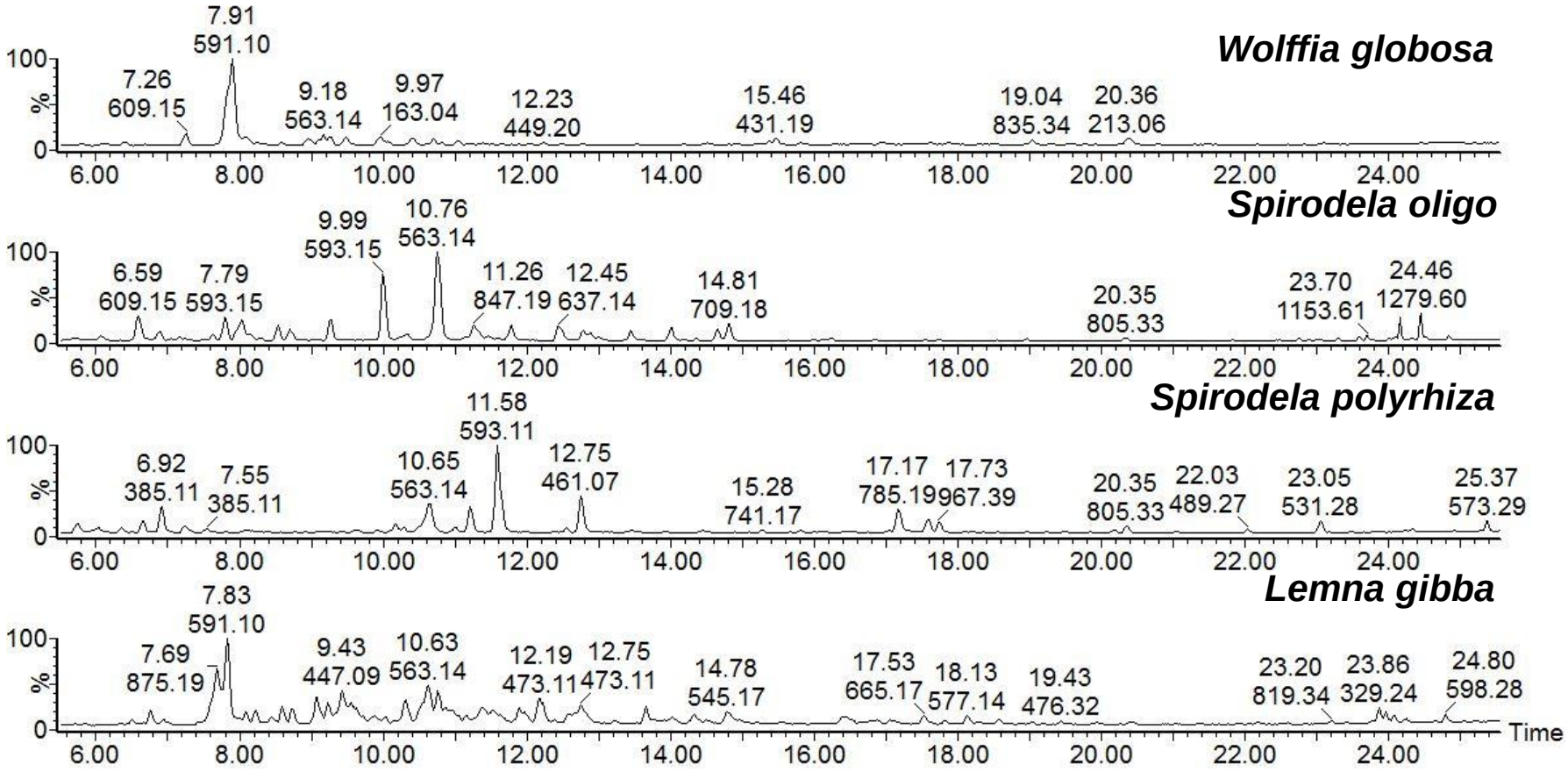


- **harvest (3 replicates) through filtration & wash with cold water**
- **homogenization with mortar & pestle (frozen)**
- **extraction in 80% MeOH + 0.1% formic acid (w/v 1:3)**
- **after filtration (0.22µm) injection on UPLC-QTOF-MS (40 min)**

- **data analysis: compound identification & validation**

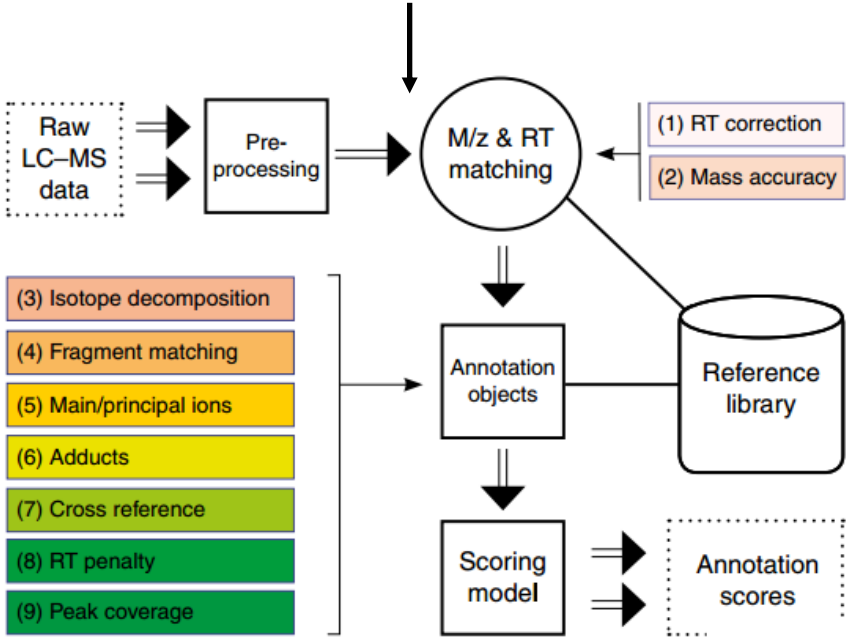
Collection screening – LC-MS (40 min gradient)

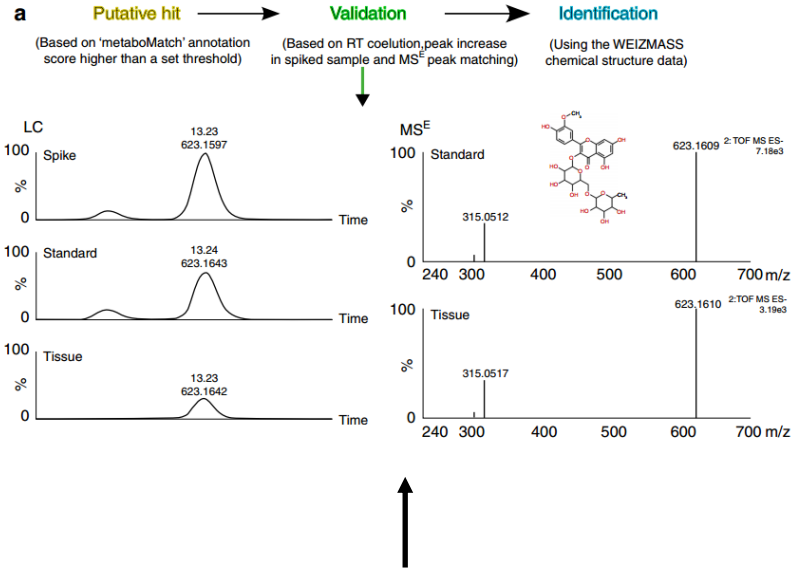
Snapshot of detected secondary metabolite diversity (6-24 min):

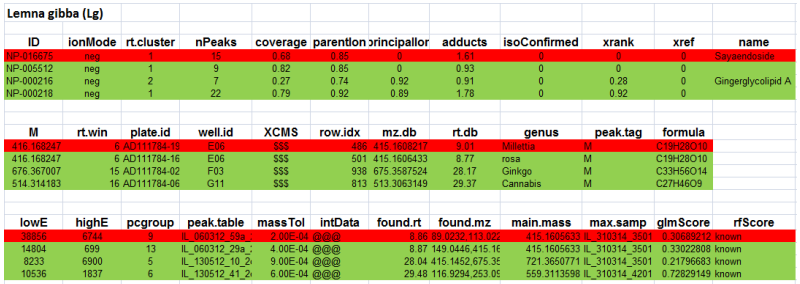


➢**data analysis strategy? --> introduction of two tools**

Weizmass/MatchWeiz – Analyticon library (3500 compounds)







Lemna gibba (Lg)

ID	ionMode	rt.cluster	nPeaks	coverage	parention	principallor	adducts	isoConfirmed	xrank	xref	name
NP-016575	neg	1	15	0.68	0.85	0	1.93	0	0	0	Sayaendoside
NP-005512	neg	1	9	0.82	0.85	0	0.93	0	0	0	
NP-000216	neg	2	7	0.27	0.74	0.92	0.91	0	0.28	0	Gingerglycolipid A
NP-000218	neg	1	22	0.79	0.92	0.89	1.78	0	0.92	0	

M	rt.win	plate.id	well.id	XCMS	row.idx	mz.db	rt.db	genus	peak.tag	formula
416.168247	6	AD111784-15	E06	$$$	486	415.1608217	9.01	Millettia	M	C19H28O10
416.168247	6	AD111784-16	E06	$$$	501	415.1606433	8.77	rosa	M	C19H28O10
676.367007	15	AD111784-02	F03	$$$	938	675.3587524	28.17	Ginkgo	M	C33H56O14
514.314183	16	AD111784-06	G11	$$$	813	513.3063149	29.37	Cannabis	M	C27H46O9

lowE	highE	pcgroup	peak.table	massTol	intData	found.rt	found.mz	main.mass	max.samp	glmScore	rfScore
38855	6744	9	IL_060312_29a_	2.00E-04	@@@	8.66	69.0232,113.022	415.1605633	IL_310314_3501	0.39688212	known
14804	699	13	IL_060312_29a_	4.00E-04	@@@	8.67	149.0446,415.16	415.1605633	IL_310314_3501	0.33022808	known
8233	6900	5	IL_130512_10_2	9.00E-04	@@@	28.04	415.1452,675.35	721.3650771	IL_310314_3501	0.21796683	known
10536	1837	6	IL_130512_41_2	6.00E-04	@@@	29.48	116.9294,253.05	559.3113598	IL_310314_4201	0.72829149	known

Figure 2 | The MatchWeiz software annotation workflow. Raw LC–MS

Shahaf et al.; Nature Communications **2016**

WEIZMANN INSTITUTE OF SCIENCE

Classyfire – module

- **software tool trained with 77 Million chemical structures for automated chemical class assignment**

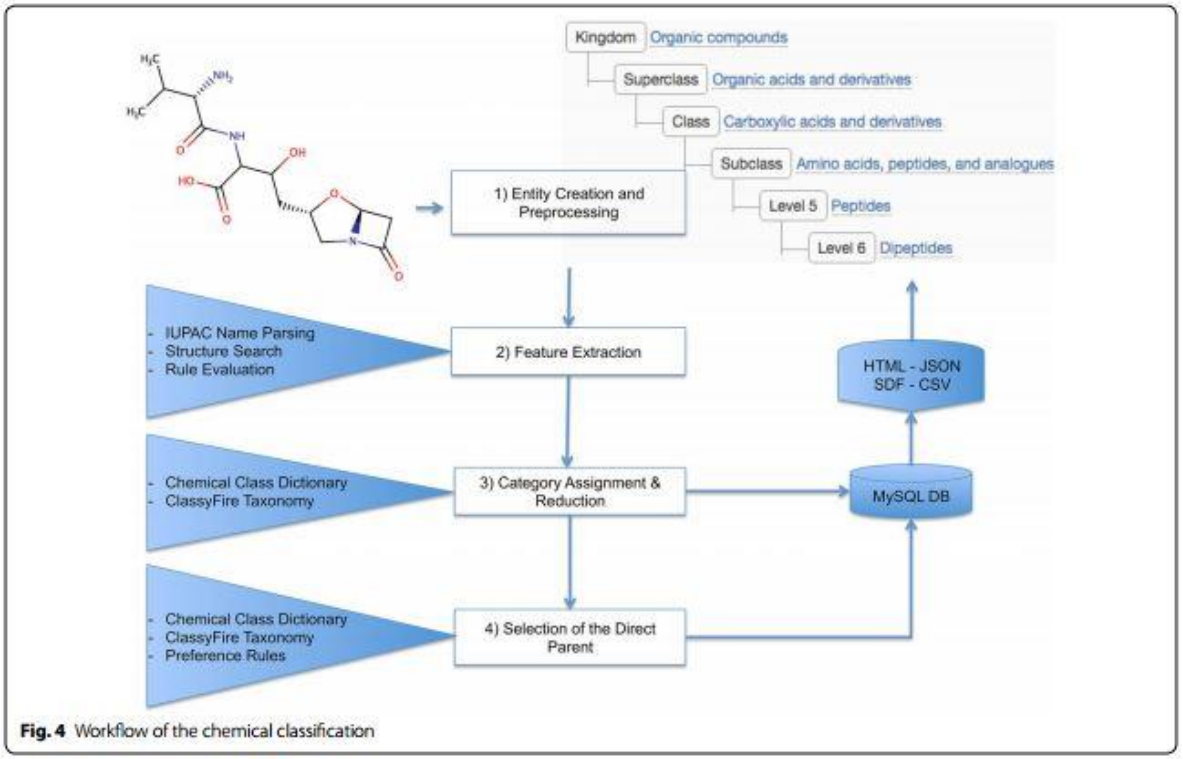
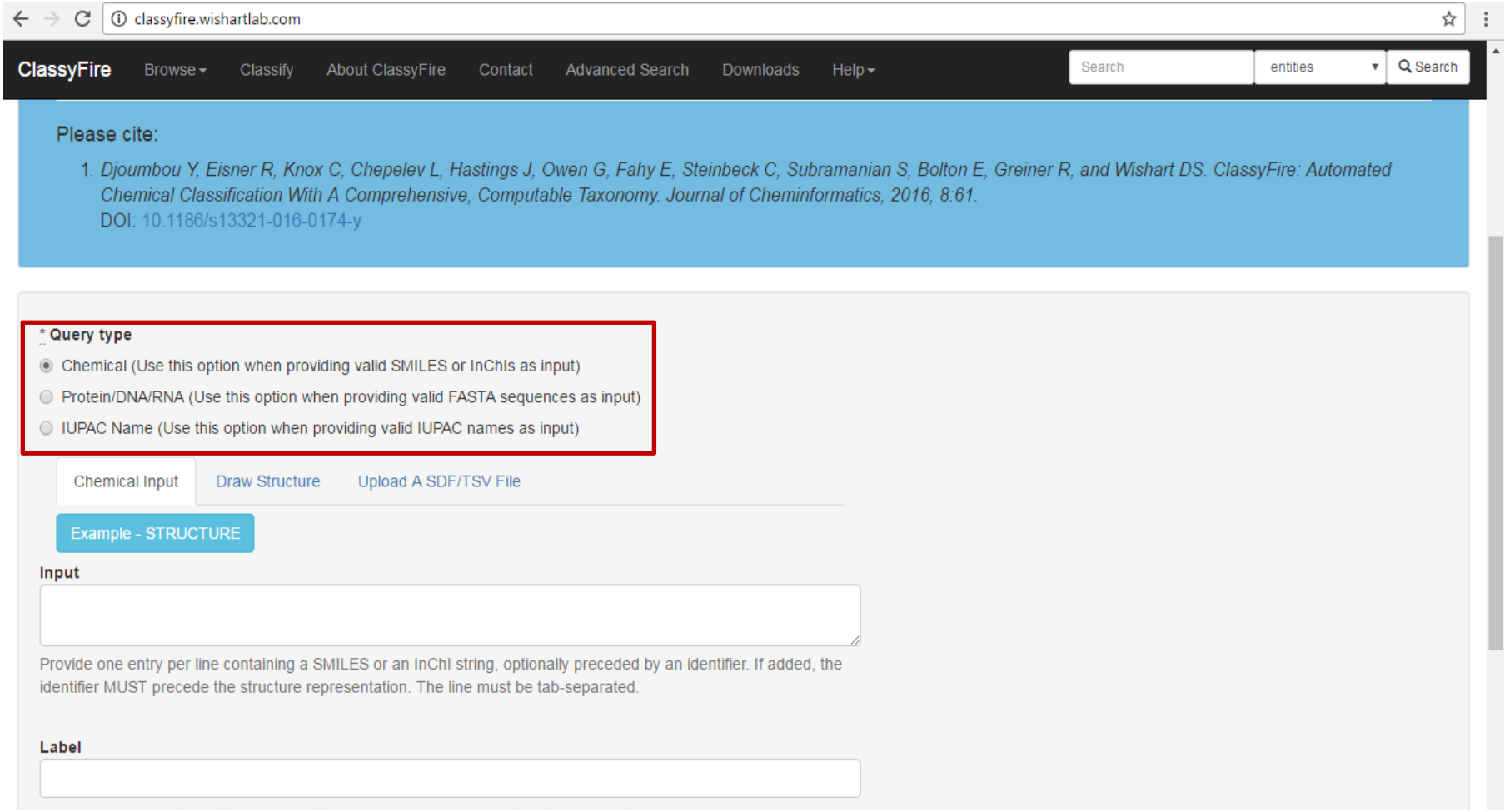


Fig. 4 Workflow of the chemical classification

Djoumbou Y, Eisner R, Knox C, Chepelev L, Hastings J, Owen G, Fahy E, Steinbeck C, Subramanian S, Bolton E, Greiner R, and Wishart DS. ClassyFire: Automated Chemical Classification With A Comprehensive, Computable Taxonomy. *Journal of Cheminformatics*, **2016**, 8:61.

WEIZMANN INSTITUTE OF SCIENCE

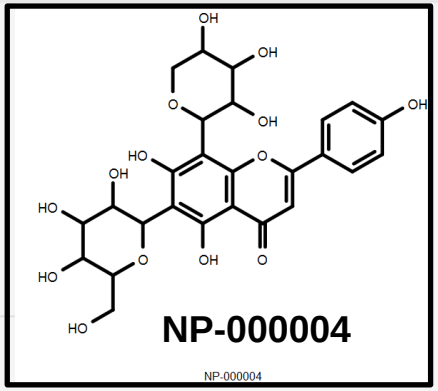
Classyfire – web server



WEIZMANN INSTITUTE OF SCIENCE

Classyfire – web server

ClassyFire | Browse | Classify | About ClassyFire | Contact | Advanced Search | Downloads | Help



*** Query type**

- (•) Chemical (Use this option when providing valid SMILES or InChIs as input)
- ( ) Protein/DNA/RNA (Use this option when providing valid FASTA sequences as input)
- ( ) IUPAC Name (Use this option when providing valid IUPAC names as input)

Chemical Input | Draw Structure | Upload A SDF/TSV File

Example - STRUCTURE

Input

"NP-000004" "[H]Oc1c([H])c([H])c(c([H])c1([H]))C=2Oc3c(C(=O)C=2([H]))c(O[H])c(c(O[H])c3C4([H])(OC([H])([H])C([H])(O[H])C([H])(O[H])C4([H])(O[H])))C5([H])(OC([H])(C([H])([H])O[H])C([H])(O[H])C([H])(O[H])C5([H])

Provide one entry per line containing a SMILES or an InChI string, optionally preceded by an identifier. If added, the identifier MUST precede the structure representation. The line must be tab-separated.

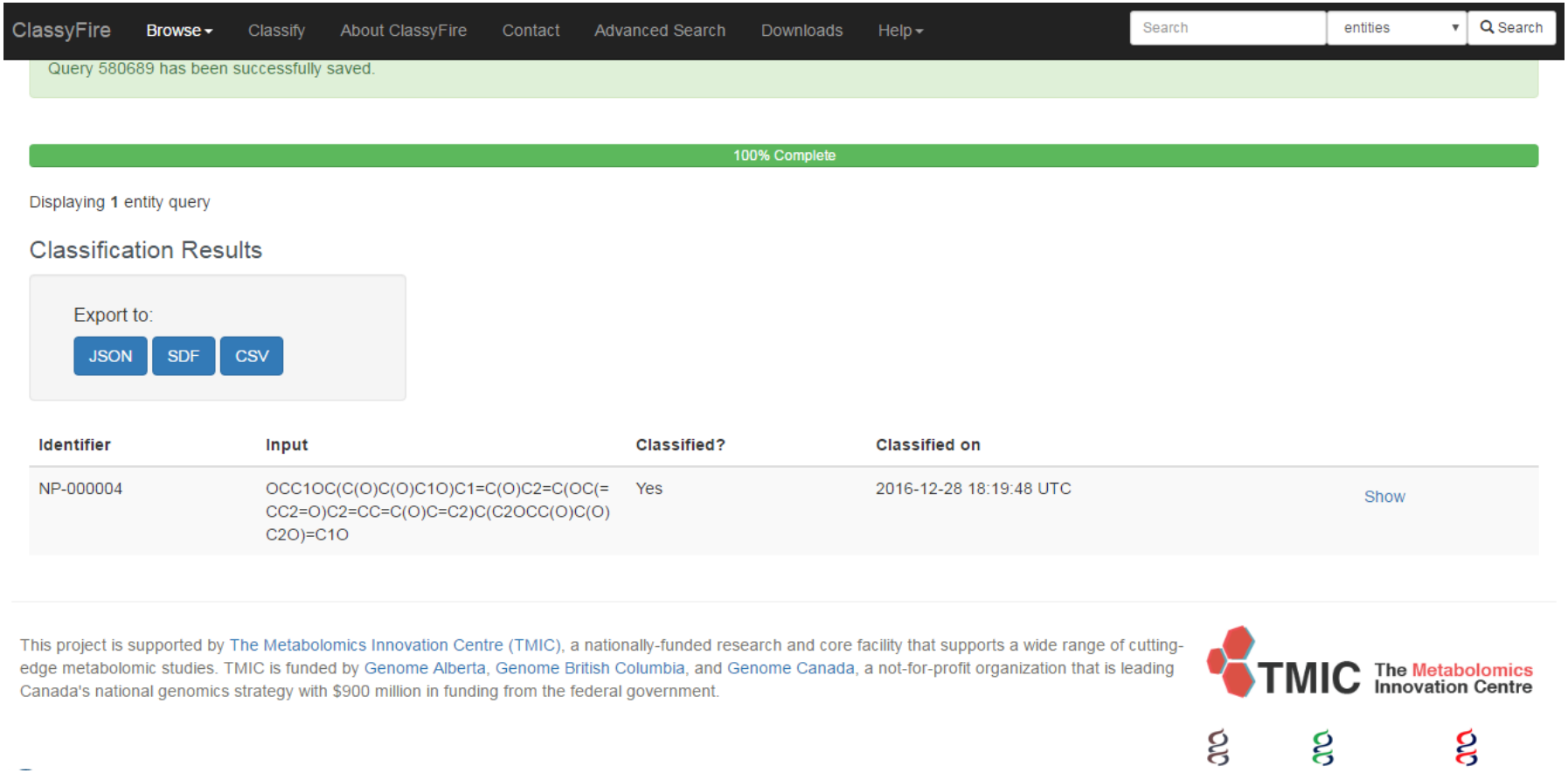
Label

Provide a name for the data sample (optional). You can provide multiple tags separated by '|'.

Submit

WEIZMANN INSTITUTE OF SCIENCE

Classyfire – web server

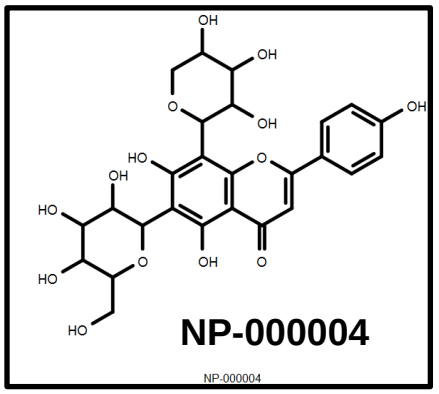


- **possibility of watching your results or exporting to sdf**
- **batch processing**

WEIZMANN INSTITUTE OF SCIENCE

Classyfire – web server

Compound Identification
SMILES
OCC1OC(C(O)C(O)C1O)C1=C(O)C2=C(OC(=CC2=O)C2=CC=C(O)C=C2)C(C2OCC(O)C(O)C2O)=C1O
InChIKey
InChIKey=MMDUKUSNQNWVET-UHFFFAOYSA-N
Formula
C26H28O14
Mass
564.496



Taxonomy Tree

- Kingdom: Organic compounds
  - Superclass: Phenylpropanoids and polyketides
    - Class: Flavonoids
      - Subclass: Flavonoid glycosides
        - Level 5: Flavonoid C-glycosides
          - Level 6: Flavonoid 8-C-glycosides

WEIZMANN INSTITUTE OF SCIENCE

Classyfire – web server

Kingdom

Organic compounds

Superclass

Phenylpropanoids and polyketides

Class

Flavonoids

Subclass

Flavonoid glycosides

Intermediate Tree Nodes

Flavonoid C-glycosides

Direct Parent

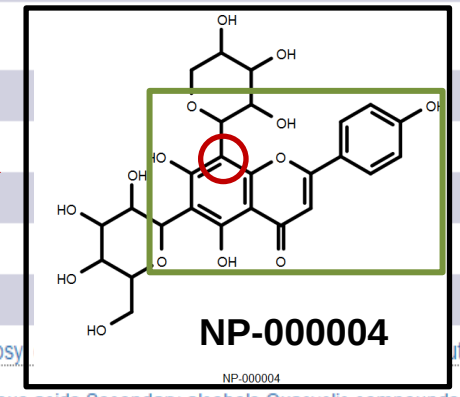
Flavonoid 8-C-glycosides

Alternative Parents

4'-hydroxyflavonoids 5-hydroxyflavonoids 7-hydroxyflavonoids Flavones Phenolic glycosides C-glycosy… …uted benzenoids Pyranones and derivatives

Monosaccharides Benzene and substituted derivatives Oxanes Heteroaromatic compounds Vinylogous acids Secondary alcohols Oxacyclic compounds Dialkyl ethers Polyols Organic oxides

Hydrocarbon derivatives Primary alcohols



- Different levels of chemical classification
- Up to 8 levels
- Number of levels depends on structure/class itself (from no classification to all levels)

- useful tool as part of metabolite identification workflow?
- Classification of WISmass library?

WEIZMANN INSTITUTE OF SCIENCE

Classyfire – Analyticon library (3500 compounds)

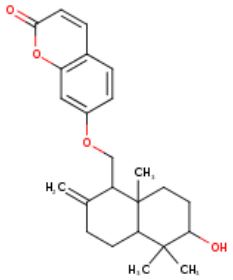
- Library was run in **Classyfire** in batches of 100 compounds (Hila)
- output as **.sdf files**
- manual **validation of 200** compounds

	A	B	C	D	E	F	G	H	I	J	K	L
1	**Classify validations**											
2					**Description:**	Meaningful levels are devided into level1: general compound class (Terpenoid, alkaloid, polyketide),						
3						level 2: subcatagory of the previous (sesquiterpene, diterpene, triterpene, steroid) & level 3: specific co						
4												
5					**Levels:**	**1**	**Kingdom**					
6						**2**	**Superclass**					
7						**3**	**Class**					
8						**4**	**Subclass**					
9						**5**	**Intermediate Nodes**					
10						**6**	**Direct parent**					
11						**7**	**alternative parents**					
12						**8**	**Molecular framework**					
13	**508505**											
14	**No**	**ID**	**correct**	**meaningful level 1**	**meaningful level 2**	**meaningful level 3**	**No. of levels**	**Uwe classy on fire delux**				
15												
16	1	NP-004636	Y	3	4	5,6	8					
17												
18	2	NP-015137	Y	2	3	/	3					
19												
20	3	NP-010695	Y	3	/	4	4					
21												
22	4	NP-002106	N				0	sesquiterpene, daucene				
23												
24	5	NP-015274	Y	2	3	3,4	4					
25												
26	6	NP-015117	Y	2	3	3,4	4					

- first meaningful level (2/3)
- # of levels (average 4-5)
- 42 wrong classifications (21%, lots of terpenes)
- what information to use?

Classyfire – "hybrid metabolites"

➢**"hybrid metabolites" are compounds that are a combination of two chemical classes**



Compound Identification
SMILES
CC1(C)C(O)CCC2(C)C(COC3=CC4=C(C=CC(=O)O4)C=C3)C(=C)CCC12
InChIKey
InChIKey=FCWYNTDTQPCVPG-UHFFFAOYSA-N
Formula
C24H30O4
Mass
382.5

Taxonomy Tree

Kingdom — Organic compounds

Superclass — Phenylpropanoids and polyketides

Class — Coumarins and derivatives

Kingdom

Organic compounds

Superclass

Phenylpropanoids and polyketides

Class

Coumarins and derivatives

Subclass

Not available

Intermediate Tree Nodes

Not available

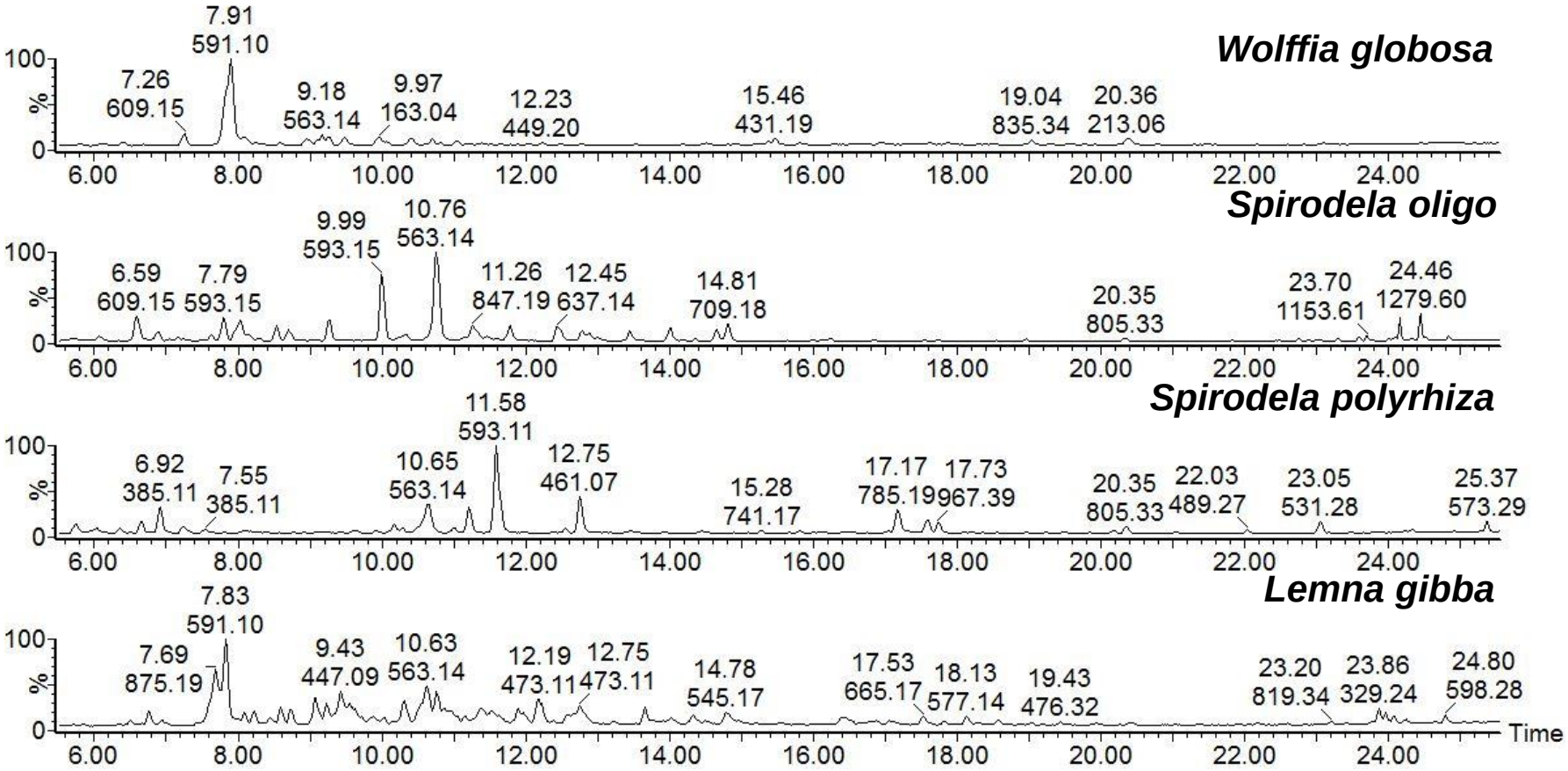
Direct Parent

Coumarins and derivatives

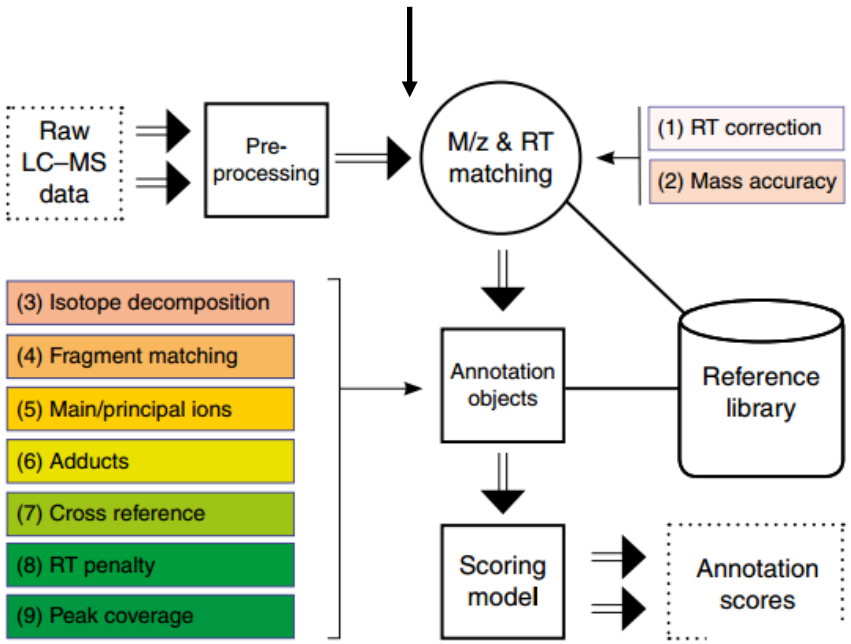
WEIZMANN INSTITUTE OF SCIENCE

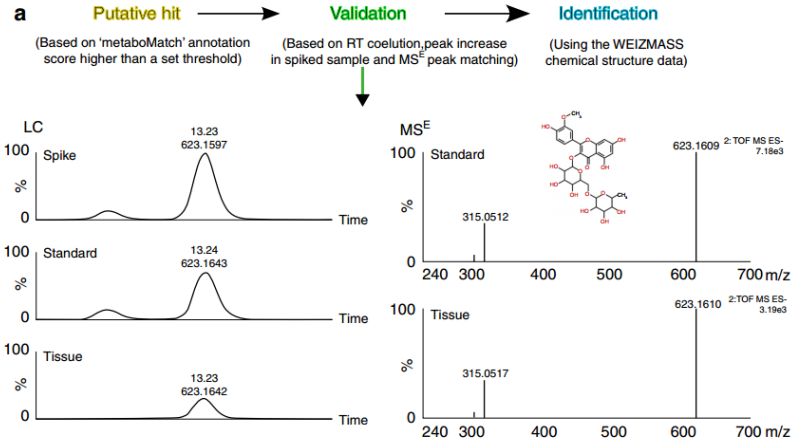
Collection screening – LC-MS (40 min gradient)

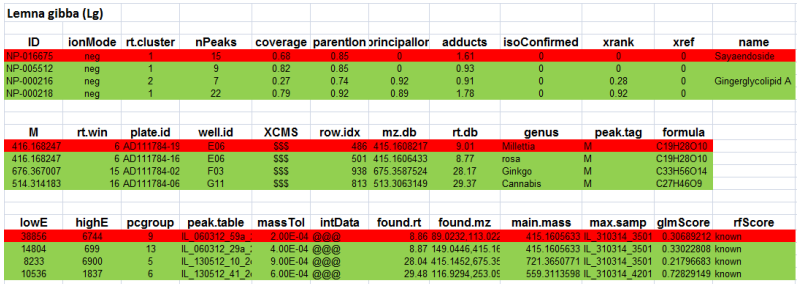
Snapshot of detected secondary metabolite diversity:



Weizmass/MatchWeiz – Analyticon library (3500 compounds)







Lemna gibba (Lg)

ID	ionMode	rt.cluster	nPeaks	coverage	parention	principallor	adducts	isoConfirmed	xrank	xref	name
NP-016575	neg	1	15	0.68	0.85	0	1.93	0	0	0	Sayaendoside
NP-005512	neg	1	9	0.82	0.85	0	0.93	0	0	0	
NP-000216	neg	2	7	0.27	0.74	0.92	0.91	0	0.28	0	Gingerglycolipid A
NP-000218	neg	1	22	0.79	0.92	0.89	1.78	0	0.92	0	

M	rt.win	plate.id	well.id	XCMS	row.idx	mz.db	rt.db	genus	peak.tag	formula
416.168247	6	AD111784-15	E06	$$$	486	415.1608217	9.01	Millettia	M	C19H28O10
416.168247	6	AD111784-16	E06	$$$	501	415.1606433	8.77	rosa	M	C19H28O10
676.367007	15	AD111784-02	F03	$$$	938	675.3587524	28.17	Ginkgo	M	C33H56O14
514.314183	16	AD111784-06	G11	$$$	813	513.3063149	29.37	Cannabis	M	C27H46O9

lowE	highE	pcgroup	peak.table	massTol	intData	found.rt	found.mz	main.mass	max.samp	glmScore	rfScore
38855	6744	9	IL_060312_29a_:	2.00E-04	@@@	8.66	69.0232,113.022	415.1605633	IL_310314_3501	0.39688212	known
14804	699	13	IL_060312_29a_:	4.00E-04	@@@	8.67	149.0446,415.16	415.1605633	IL_310314_3501	0.33022808	known
8233	6900	5	IL_130512_10_2	9.00E-04	@@@	28.04	415.1452,675.35	721.3650771	IL_310314_3501	0.21796683	known
10536	1837	6	IL_130512_41_2	6.00E-04	@@@	29.48	116.9294,253.05	559.3113598	IL_310314_4201	0.72829149	known

Figure 2 | The MatchWeiz software annotation workflow. Raw LC–MS

Shahaf , Rogachev, Heinig et al.; Nature Communications **2016**

WEIZMANN INSTITUTE OF SCIENCE

Collection screening – Weizmass/MatchWeiz

- Due to diversity of the samples **pre-processing** was performed **per genus** (***Lemna+Landoltia, Wolffiella, Spirodela, Wolffia***)
- individual processing with **Weizmass/MatchWeiz**
- **initial identification tables per genus**
- comparison of **common hits** between groups and identification of **previously identified compounds** (Shahaf et al. 2016)

Lemna gibba (Lg)

ID	ionMode	rt.cluster	nPeaks	coverage	parentIon	principalIor	adducts	isoConfirmed	xrank	xref	name
NP-016675	neg	1	15	0.68	0.85	0	1.61	0	0	0	Sayaendoside
NP-005512	neg	1	9	0.82	0.85	0	0.93	0	0	0	
NP-000216	neg	2	7	0.27	0.74	0.92	0.91	0	0.28	0	Gingerglycolipid A
NP-000218	neg	1	22	0.79	0.92	0.89	1.78	0	0.92	0	

M	rt.win	plate.id	well.id	XCMS	row.idx	mz.db	rt.db	genus	peak.tag	formula
416.168247	6	AD111784-19	E06	$$$	486	415.1608217	9.01	Millettia	M	C19H28O10
416.168247	6	AD111784-16	E06	$$$	501	415.1606433	8.77	rosa	M	C19H28O10
676.367007	15	AD111784-02	F03	$$$	938	675.3587524	28.17	Ginkgo	M	C33H56O14
514.314183	16	AD111784-06	G11	$$$	813	513.3063149	29.37	Cannabis	M	C27H46O9

lowE	highE	pcgroup	peak.table	massTol	intData	found.rt	found.mz	main.mass	max.samp	glmScore	rfScore
38856	6744	9	IL_060312_59a_	2.00E-04	@@@	8.86	89.0232,113.022	415.1605633	IL_310314_3501	0.30689212	known
14804	699	13	IL_060312_29a_	4.00E-04	@@@	8.87	149.0446,415.16	415.1605633	IL_310314_3501	0.33022808	known
8233	6900	5	IL_130512_10_2	9.00E-04	@@@	28.04	415.1452,675.35	721.3650771	IL_310314_3501	0.21796683	known
10536	1837	6	IL_130512_41_2	6.00E-04	@@@	29.48	116.9294,253.09	559.3113598	IL_310314_4201	0.72829149	known

Collection screening – MatchWeiz compound validation

- **Spiking of compound in sample with highest intensity of compound**
- **only in one extract (genus)**

Know in Lemna, not spiked

In Lemna & Spirodela, spiked in Lemna

Lemna	Spirodela	Wolffia	Woolffiela	common
*NP-001271	*NP-000835	*NP-000002	NP-004596	**NP-000212
*NP-000286	NP-016821	*NP-001271	**NP-000061	NP-004940
*NP-000002	*NP-004586	*NP-000286	NP-015651	NP-005064
*NP-000287	*NP-007187	**NP-000212	NP-002437	*NP-004586
NP-013345	NP-001730	**NP-000437	NP-001362	NP-016976
NP-001730	NP-004573	NP-003191	NP-016976	NP-001009
NP-005587	**NP-000437	*NP-007187	NP-017063	**NP-000887
NP-003191	NP-003191	NP-001730	NP-004940	NP-000427
NP-000588	NP-005155	*NP-000835	*NP-004586	*NP-010155
**NP-000212	NP-000902	*NP-000287	NP-002465	NP-015231
NP-000895	**NP-000212	NP-001393	*NP-002970	NP-015334
NP-015692	*NP-000836	NP-000588	NP-005332	NP-017147
*NP-001984	*NP-013098	NP-000062	NP-008402	**NP-005379

Common in all, known from other species, spiked in Wolffiella

- **injection of spike, sample and pure standard -> identification**

Collection screening – MatchWeiz compound validation

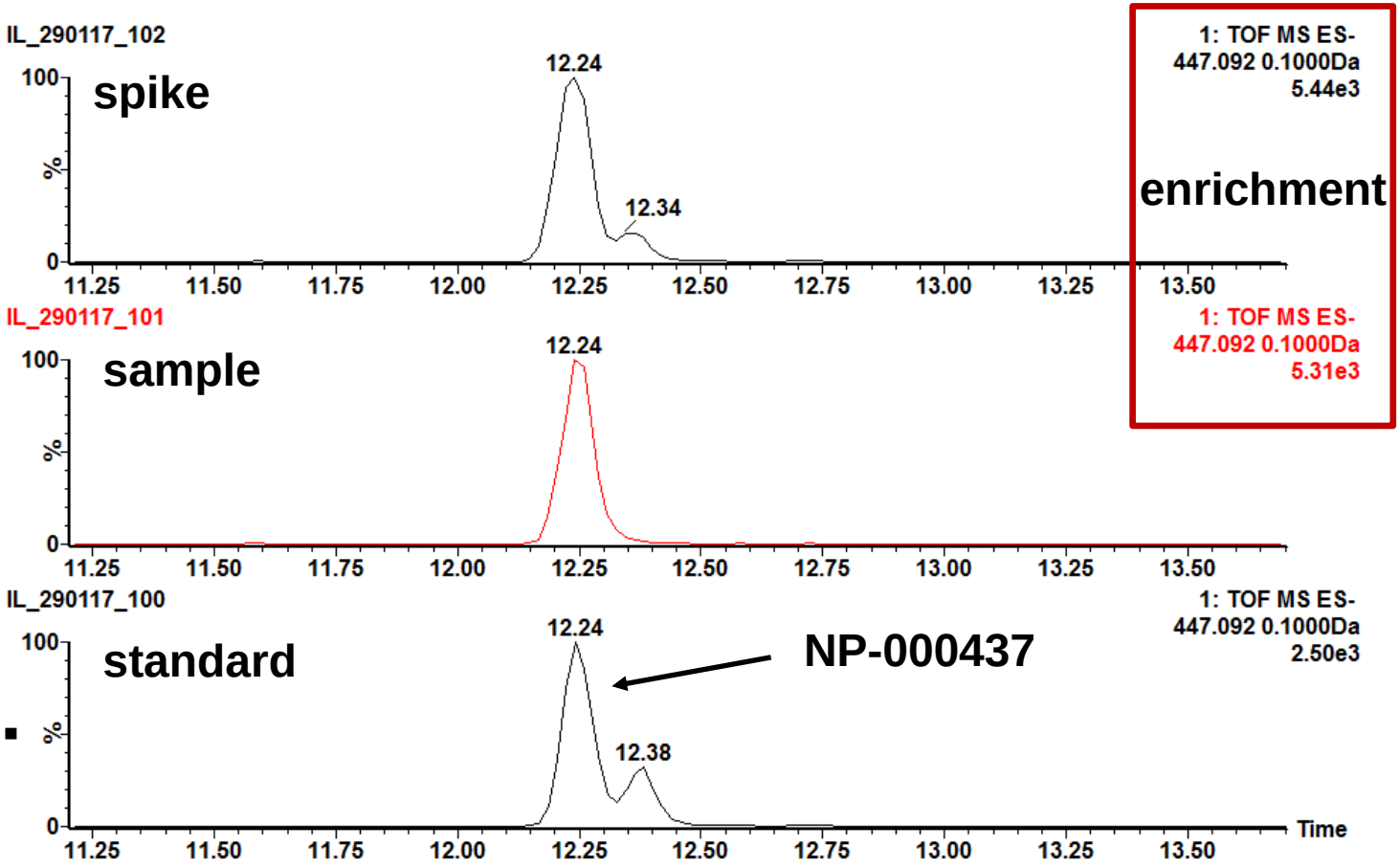
➢ **Possible results of spiking experiment:**

- **positive identification (RT, MS, MS fragmentation identical)**
- **related = false positive (wrong RT or mass)**
- **wrong identification (no criterium matches)**
- **identification of isomers through impurities in the standard (same mass, MS fragmentation, different RT)**

WEIZMANN INSTITUTE OF SCIENCE

Collection screening – MatchWeiz compound validation

➢ positive identification ESI- (88 compounds)

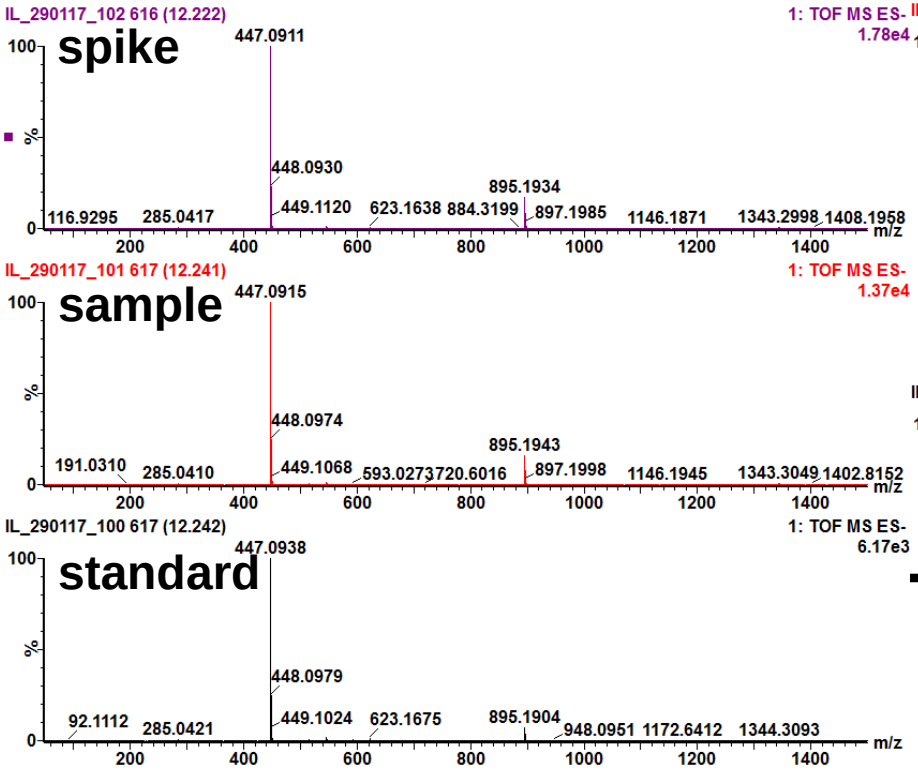


WEIZMANN INSTITUTE OF SCIENCE

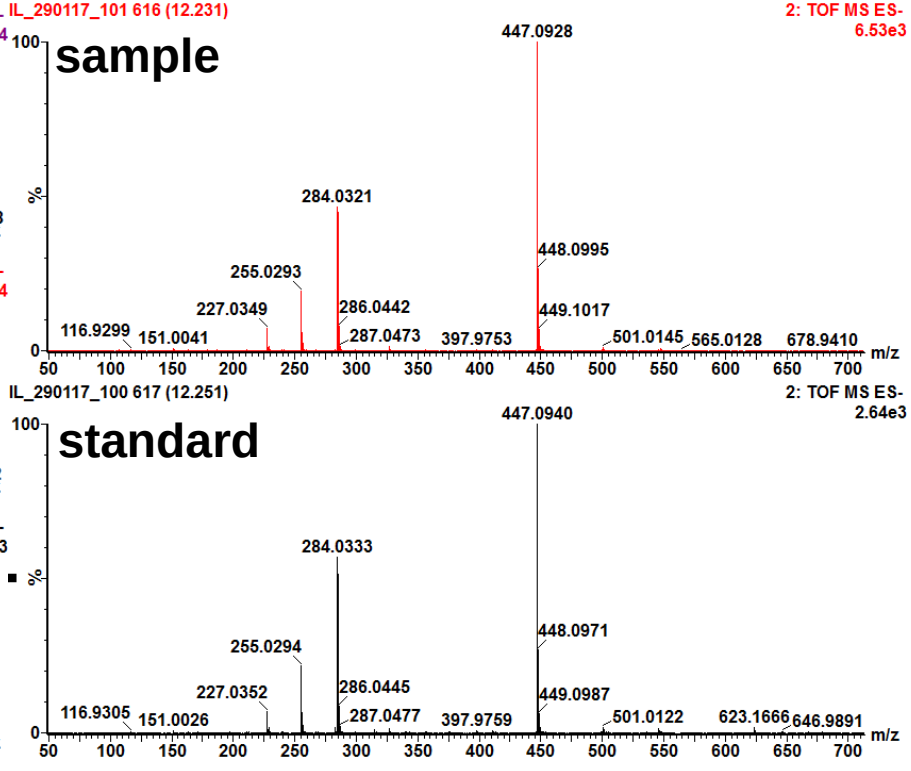
Collection screening – MatchWeiz compound validation

➢ **positive identification (88 compounds)**

MS



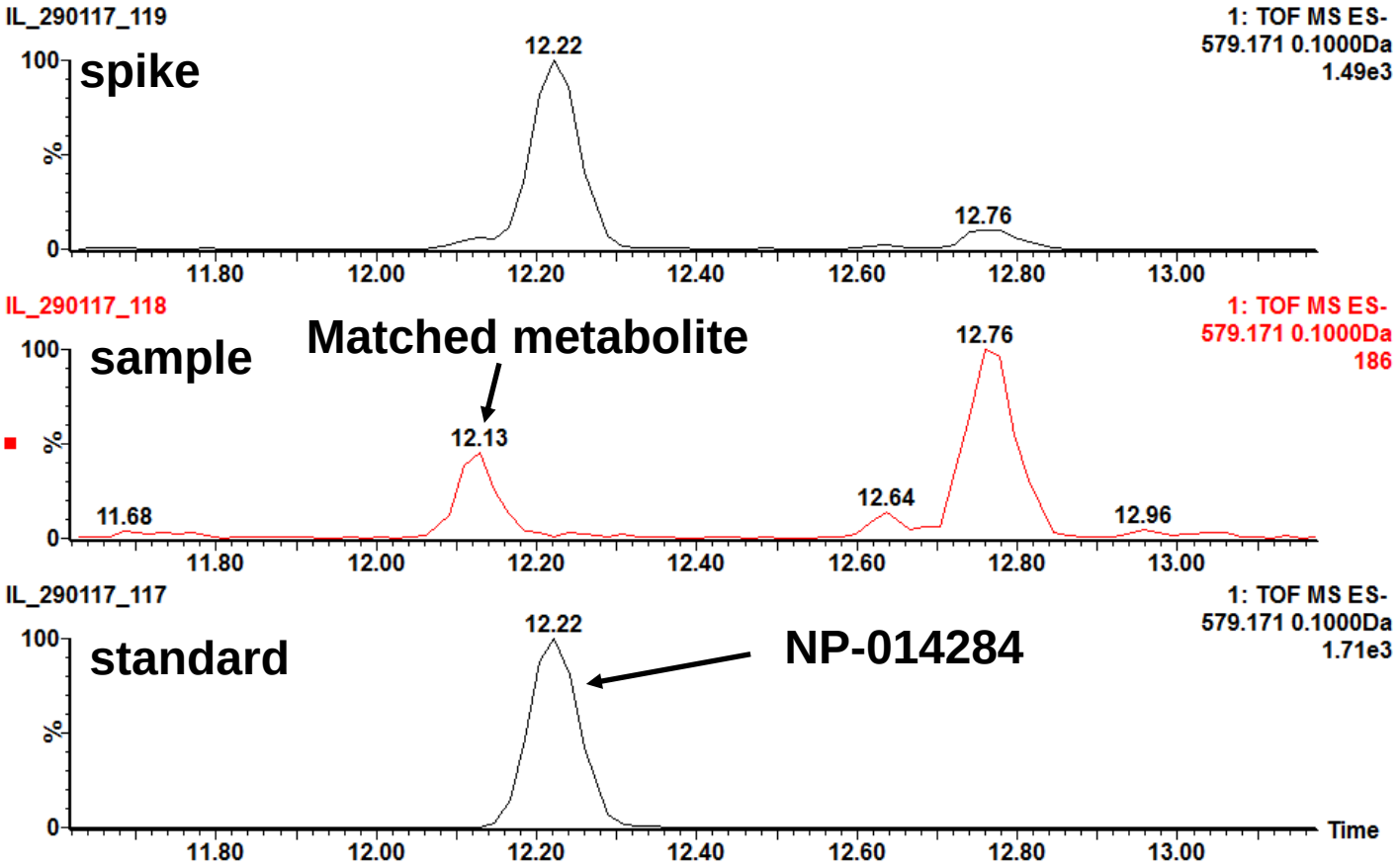
MSramp



WEIZMANN INSTITUTE OF SCIENCE

Collection screening – MatchWeiz compound validation

- **related compound = false positive (Most common case, RT difference sometimes very small, 0.02 min)**



WEIZMANN INSTITUTE OF SCIENCE

Collection screening – MatchWeiz compound validation

- **compounds that are “too similar”**

Lemna gibba (Lg)

ID	ionMode	rt.cluster	nPeaks	coverage	parentIon	principalIor	adducts	isoConfirmed	xrank	xref	name
NP-016675	neg	1	15	0.68	0.85	0	1.61	0	0	0	Sayaendoside
NP-005512	neg	1	9	0.82	0.85	0	0.93	0	0	0	
NP-000216	neg	2	7	0.27	0.74	0.92	0.91	0	0.28	0	Gingerglycolipid A
NP-000218	neg	1	22	0.79	0.92	0.89	1.78	0	0.92	0	

M	rt.win	plate.id	well.id	XCMS	row.idx	mz.db	rt.db	genus	peak.tag	formula
416.168247	6	AD111784-19	E06	$$$	486	415.1608217	9.01	Millettia	M	C19H28O10
416.168247	6	AD111784-16	E06	$$$	501	415.1606433	8.77	rosa	M	C19H28O10
676.367007	15	AD111784-02	F03	$$$	938	675.3587524	28.17	Ginkgo	M	C33H56O14
514.314183	16	AD111784-06	G11	$$$	813	513.3063149	29.37	Cannabis	M	C27H46O9

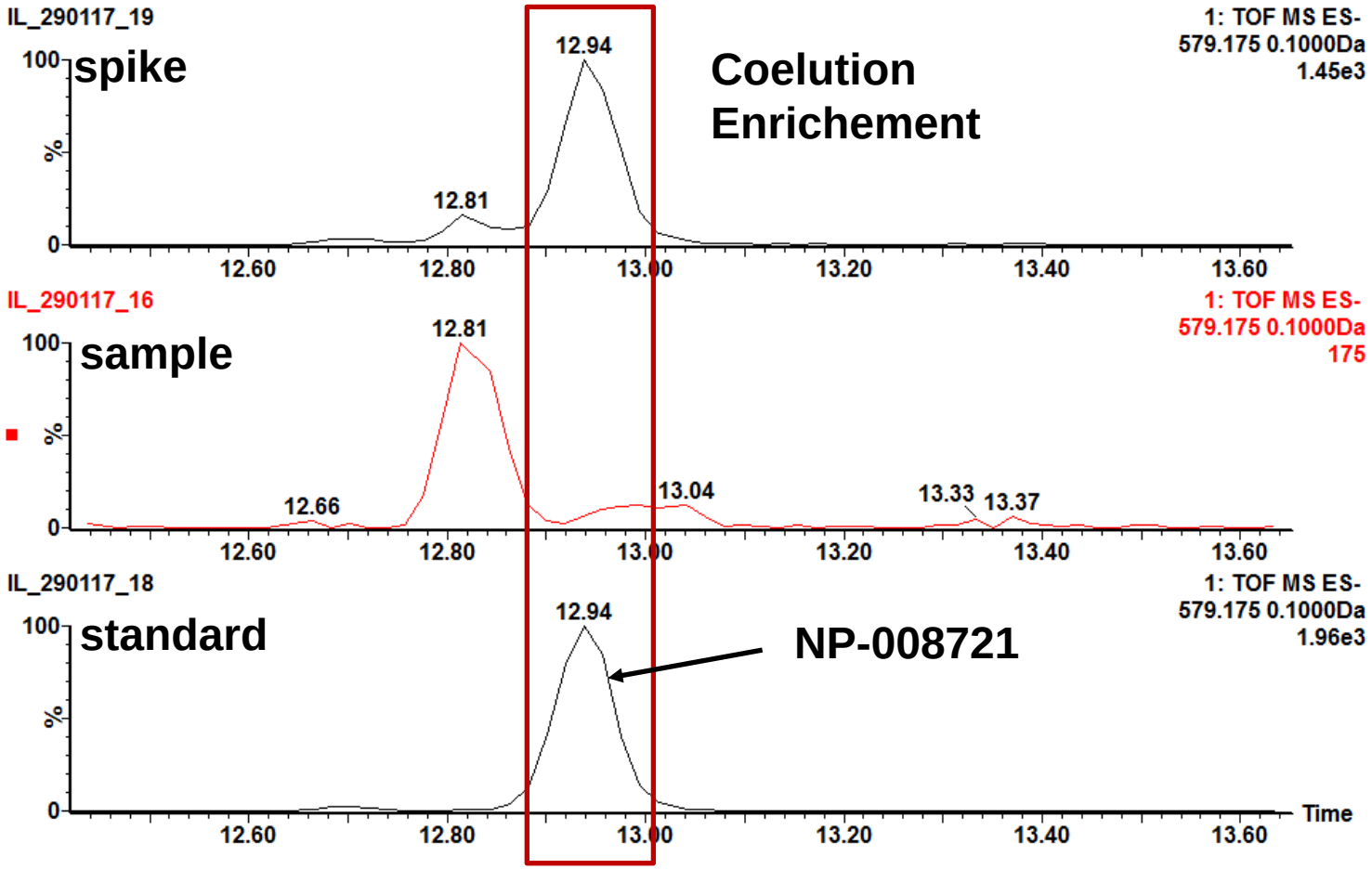
lowE	highE	pcgroup	peak.table	massTol	intData	found.rt	found.mz	main.mass	max.samp	glmScore	rfScore
38856	6744	9	IL_060312_59a_	2.00E-04	@@@	8.86	89.0232,113.022	415.1605633	IL_310314_3501	0.30689212	known
14804	699	13	IL_060312_29a_	4.00E-04	@@@	8.87	149.0446,415.16	415.1605633	IL_310314_3501	0.33022808	known
8233	6900	5	IL_130512_10_2	9.00E-04	@@@	28.04	415.1452,675.35	721.3650771	IL_310314_3501	0.21796683	known
10536	1837	6	IL_130512_41_2	6.00E-04	@@@	29.48	116.9294,253.09	559.3113598	IL_310314_4201	0.72829149	known

- **one compound is true one false, only by spiking !**

WEIZMANN INSTITUTE OF SCIENCE

Collection screening – MatchWeiz compound validation

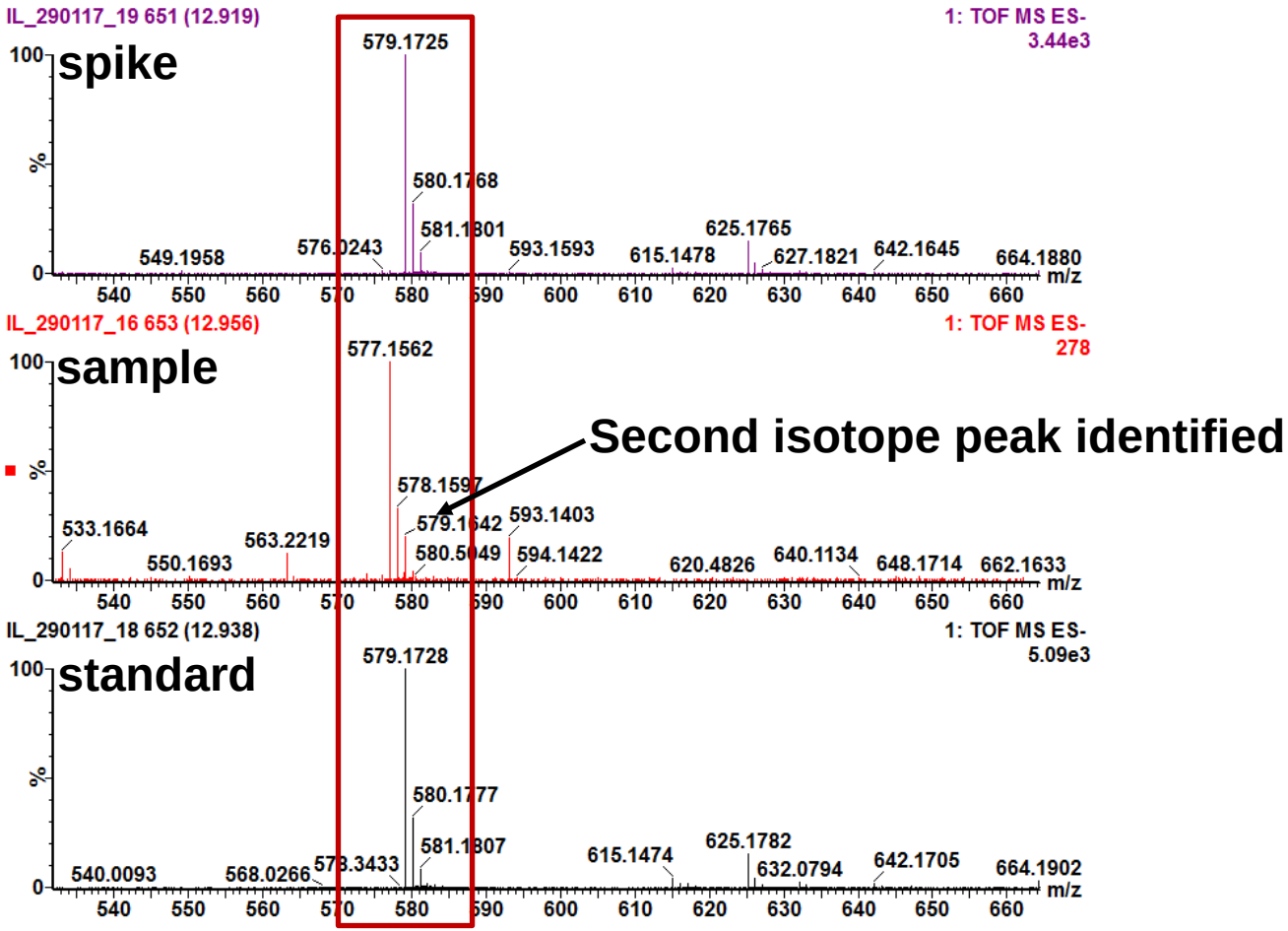
➢**library compound corresponds to isotope of sample compound**



WEIZMANN INSTITUTE OF SCIENCE

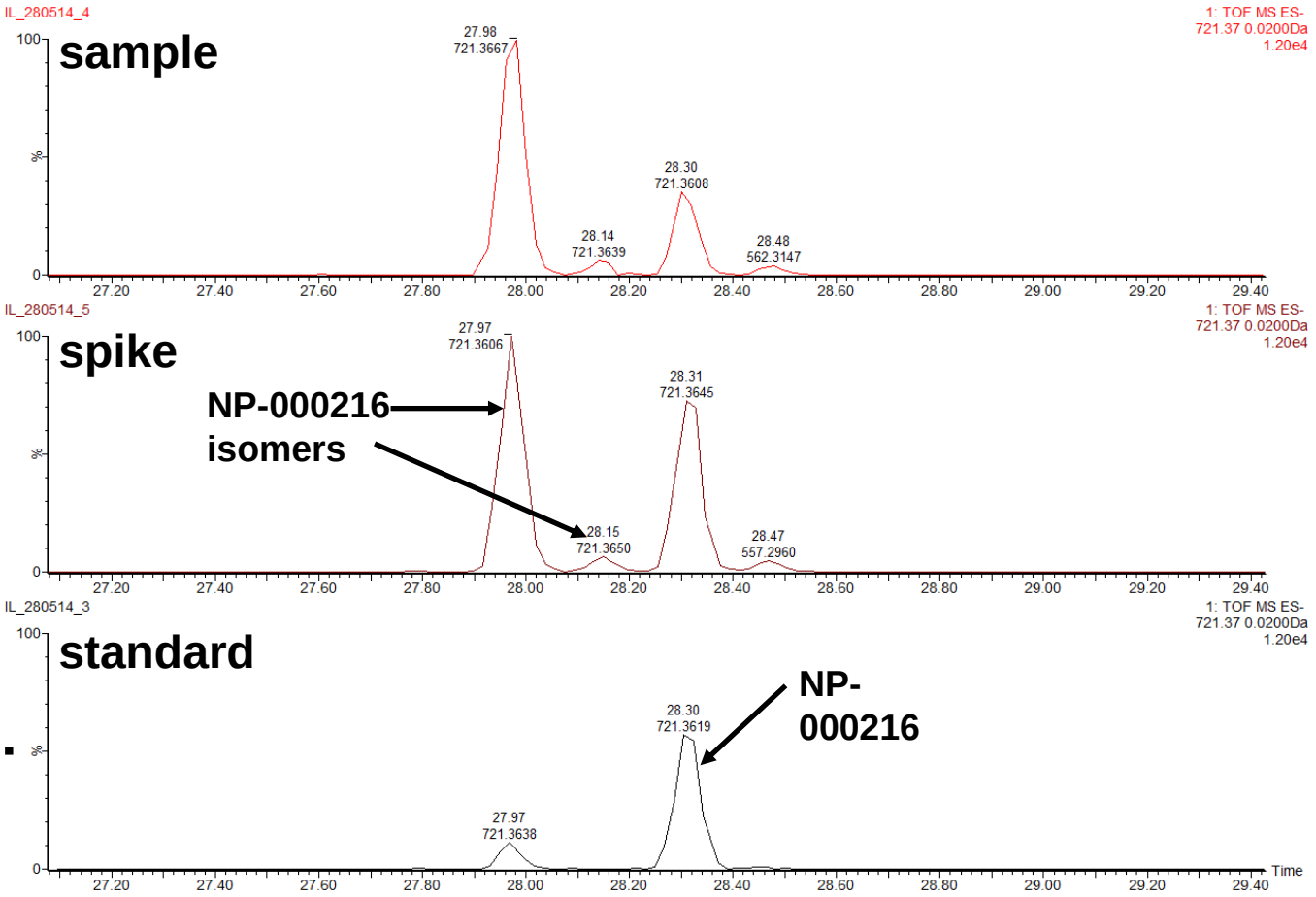
Collection screening – MatchWeiz compound validation

➢**library compound corresponds to isotope of sample compound**



Collection screening – MatchWeiz compound validation

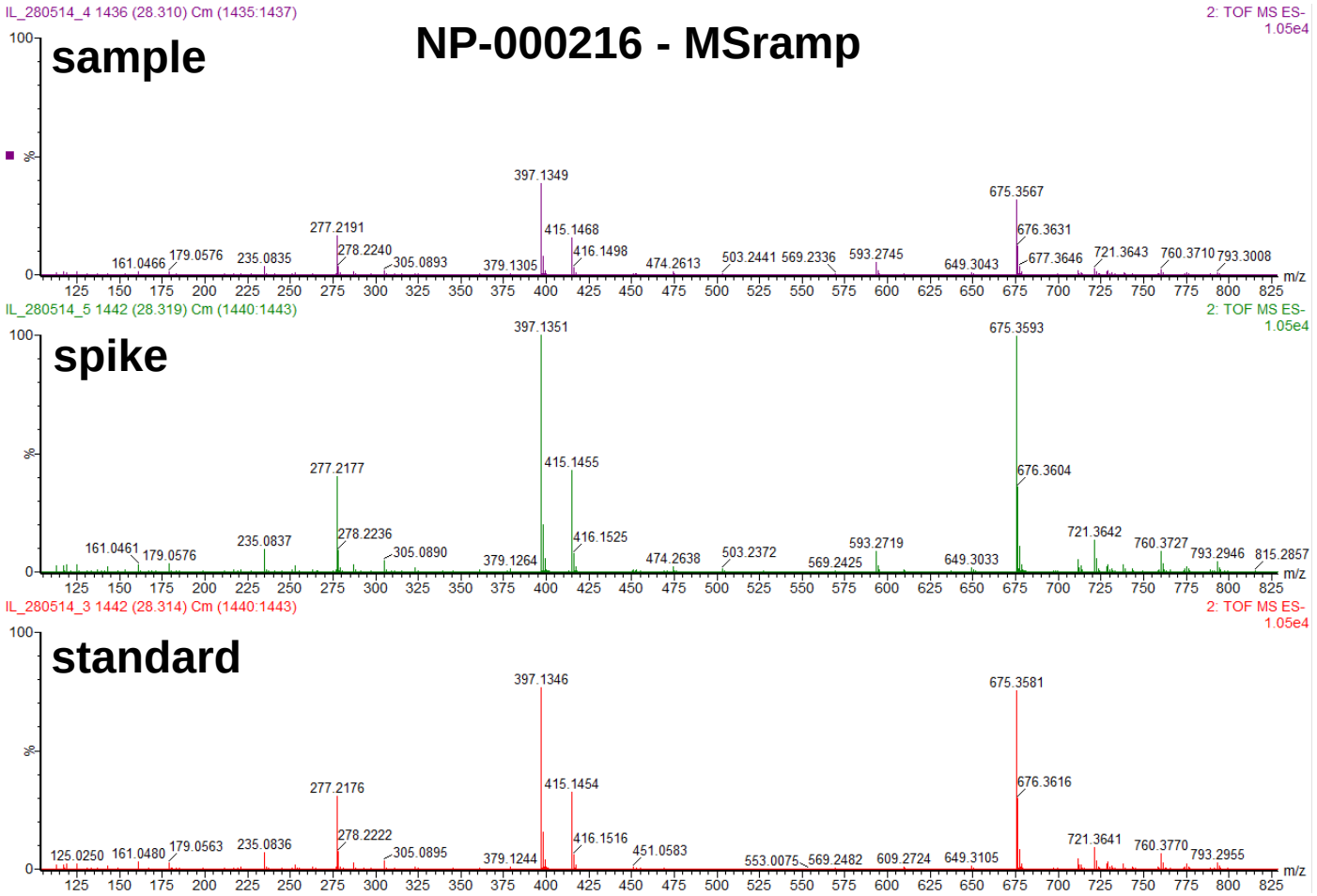
- **identification of isomers (many times present in standard, more than 15)**



WEIZMANN INSTITUTE OF SCIENCE

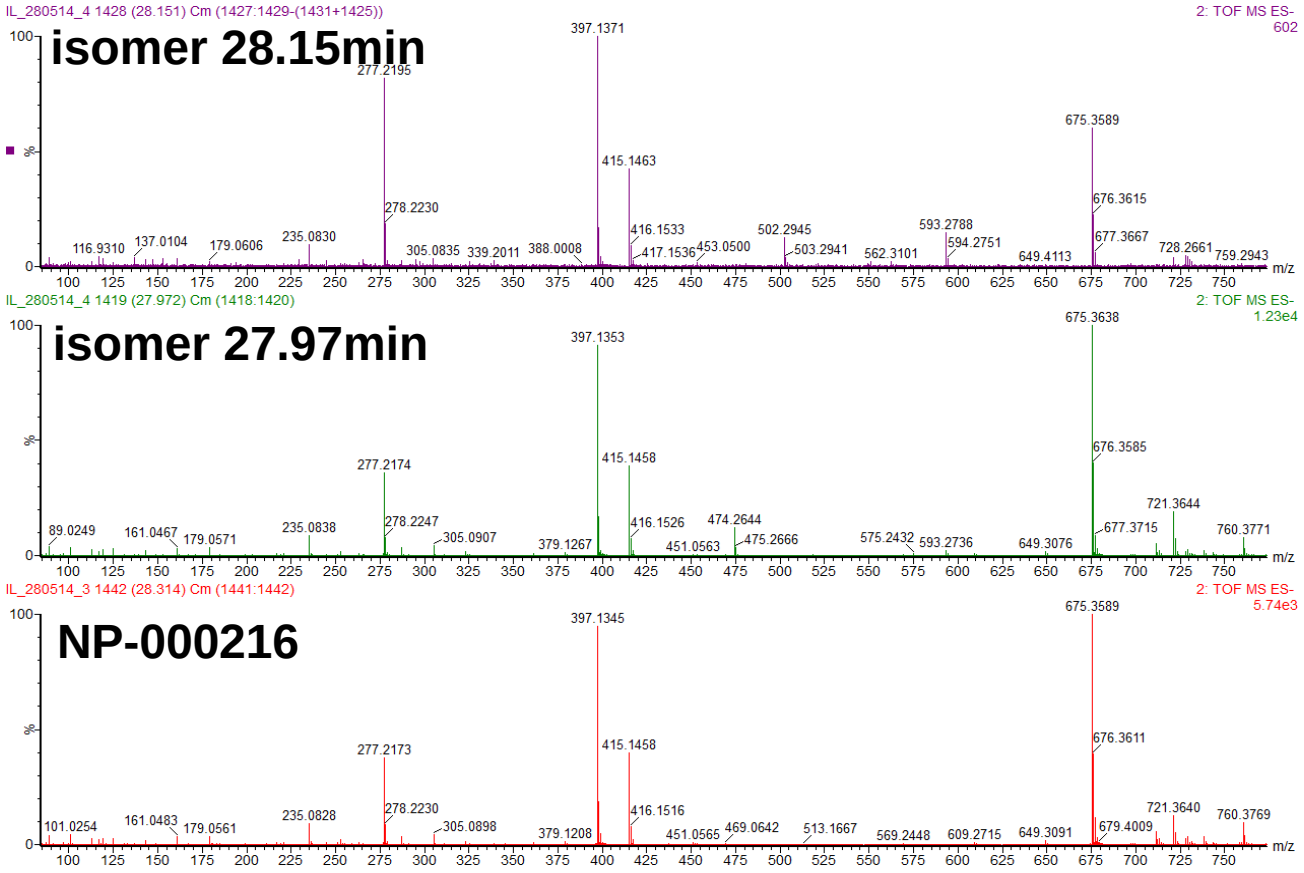
Collection screening – MatchWeiz compound validation

➢**identification of isomers (many times present in standard, more than 15)**



Collection screening – MatchWeiz compound validation

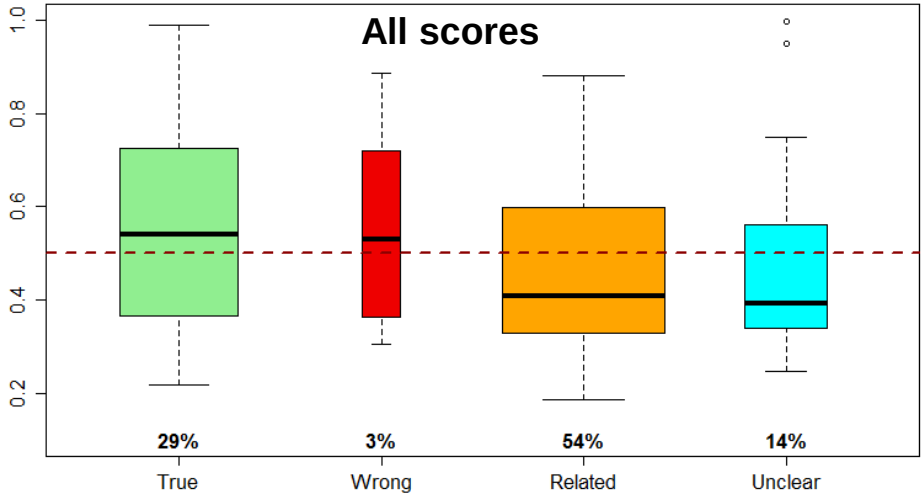
- **identification of isomers (many times present in standard, more than 15)**

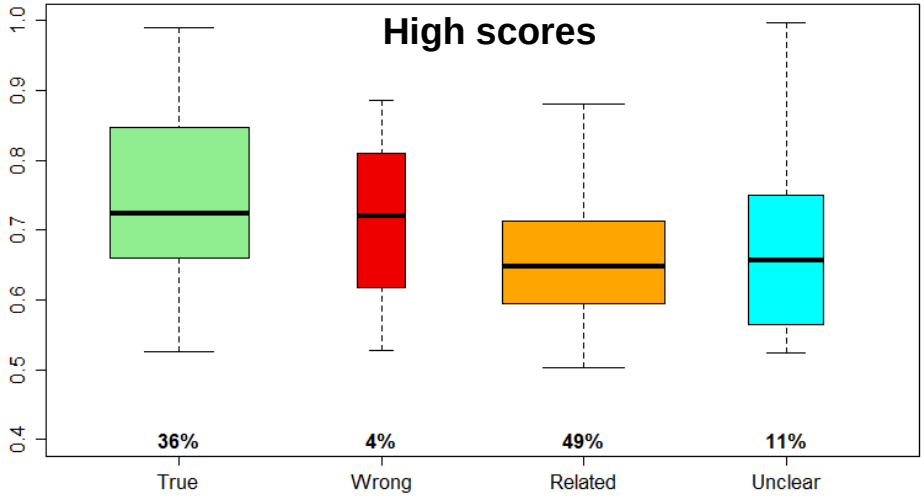


WEIZMANN INSTITUTE OF SCIENCE

Collection screening – MatchWeiz compound validation

➢ **88 positive identifications (+15 isomers)**



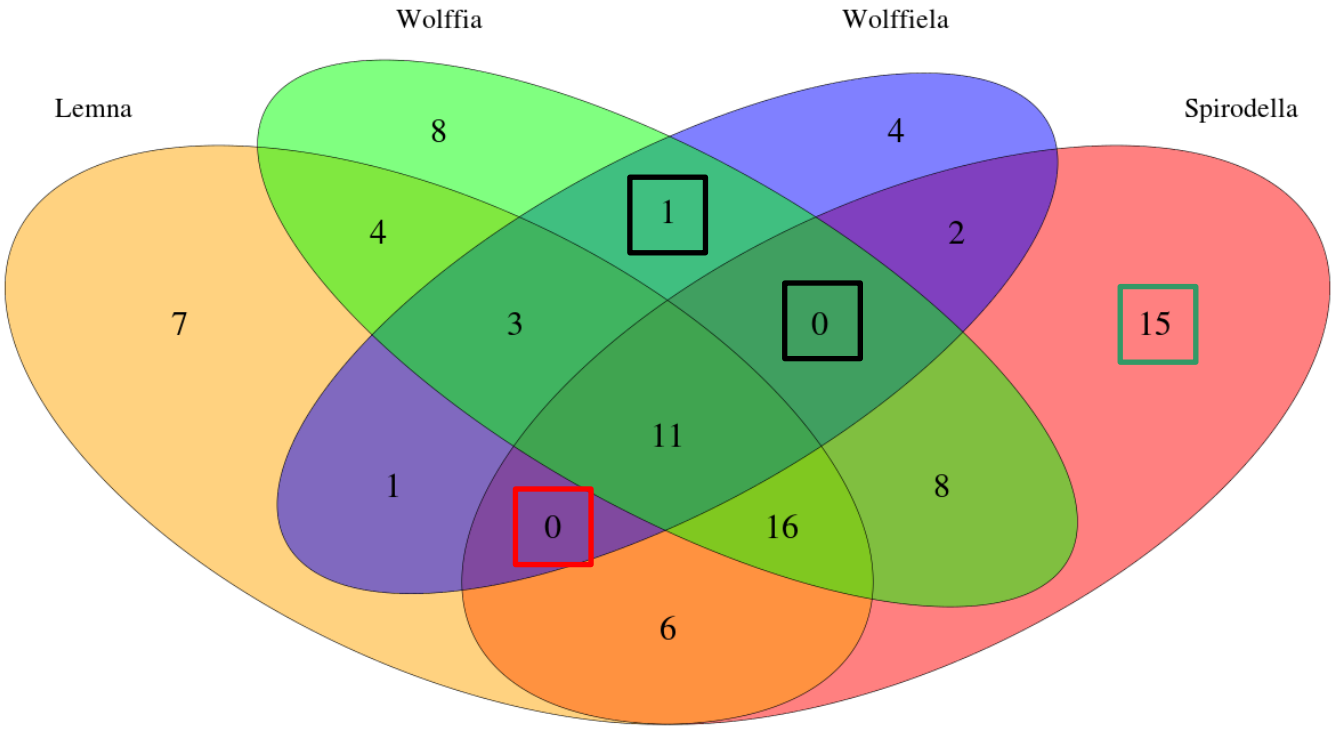


➢ **around 30% positive identifications (+isomers)**

➢ **unclear compounds need MS-MS analysis**

➢ **cut off by score is problematic and leads to loss of identifications**

Collection screening – analysis of Lemna metabolites



- ***Spirodela** has most unique metabolites (although only two species)*
- ***Although Wolffia and Wolffiella** are related they share only one compound*

WEIZMANN INSTITUTE OF SCIENCE

Collection screening – analysis of Lemna metabolites

➢Classification of Lemnaceae compounds (88)

5 classes general

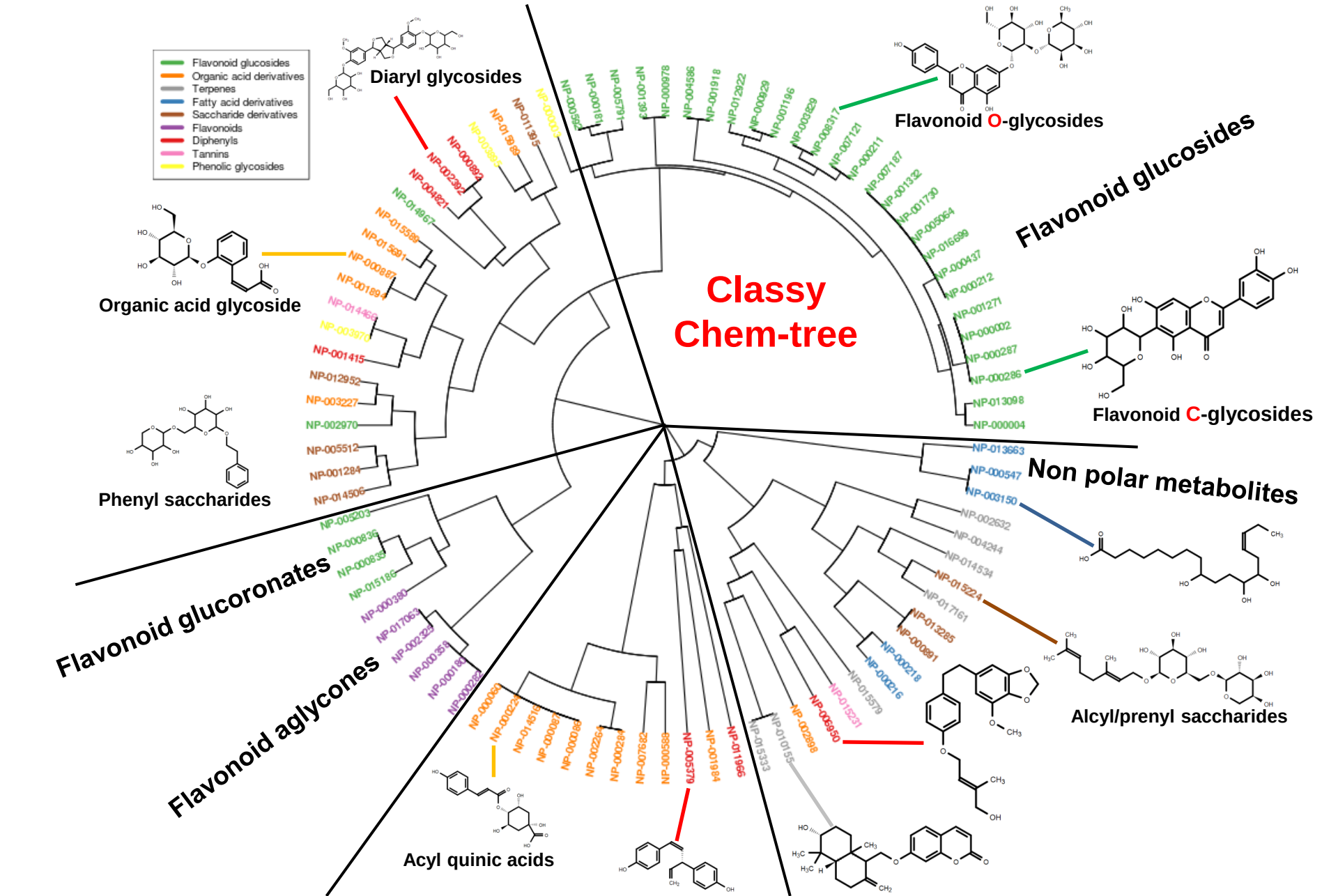
ID positive hit	classyfire 1st	classyfire 2nd	classyfire 3rd	correct	Uwe
NP-000004	Phenylpropanoids	Flavonoid glucoside	Flavonoid C-glucoside	Y	N
NP-000286	Phenylpropanoids	Flavonoid glucoside		Y	N
NP-000060	Phenylpropanoids	Organic acid derivatives	Coumaroylquinic acid	N	Y
NP-015333	Terpene	Sesquiterpene	Coumarin-Sesquiterpene conjugate	N	Y
	Phenylpropanoids	Coumarins	Coumarin derivatives	Y	Y
NP-000211	Phenylpropanoids	Flavonoid glucoside	Flavonoid O-glucoside	Y	N
NP-005203	Phenylpropanoids	Flavonoid glucoside	Flavonoid O-glucoside	Y	N
NP-000216	Lipids	Glycerolipids	Glucosylacylglycerols	Y	Y
NP-000212	Phenylpropanoids	Flavonoid glucoside	Flavonoid O-glucoside	Y	N
NP-011395	Carbohydrate conjugates	Benzyl-saccharide	Benzyl-disaccaride	Y	Y
NP-000437	Phenylpropanoids	Flavonoid glucoside	Flavonoid O-glucoside	N	Y
NP-016699	Phenylpropanoids	Flavonoid glucoside	Flavonoid O-glucoside	N	Y
NP-005064	Phenylpropanoids	Flavonoid glucoside	Flavonoid O-glucoside	N	Y
NP-015589	Phenylpropanoids	Organic acid derivatives	Ferulic acid O-glycoside	N	Y
NP-003150	Lipids	Fatty acyls	Hydroxy fatty acid	Y	Y
NP-005791	Phenylpropanoids	Flavonoid glucoside	Flavonoid C-glucoside	Y	N
NP-007121	Phenylpropanoids		Flavonoid O-glucoside	Y	N
NP-012952	Carbohydrate conjugate		Catechol	Y	Y
NP-017063	Phenylpropanoids	Flavonoids	Flavans	Y	N
NP-001730	Phenylpropanoids	Flavonoid glucoside	Flavonoid O-glucoside	N	Y
NP-000181	Phenylpropanoids	Flavonoid glucoside	Flavonoid O-glucoside	N	Y
NP-005512	Carbohydrate conjugates	Benzyl-saccharide	Benzyl-disaccharide	Y	Y
NP-010155	Terpene	Sesquiterpene	Coumarin-Sesquiterpene conjugate	N	Y
	Phenylpropanoids	Coumarins	Coumarin derivatives	Y	Y

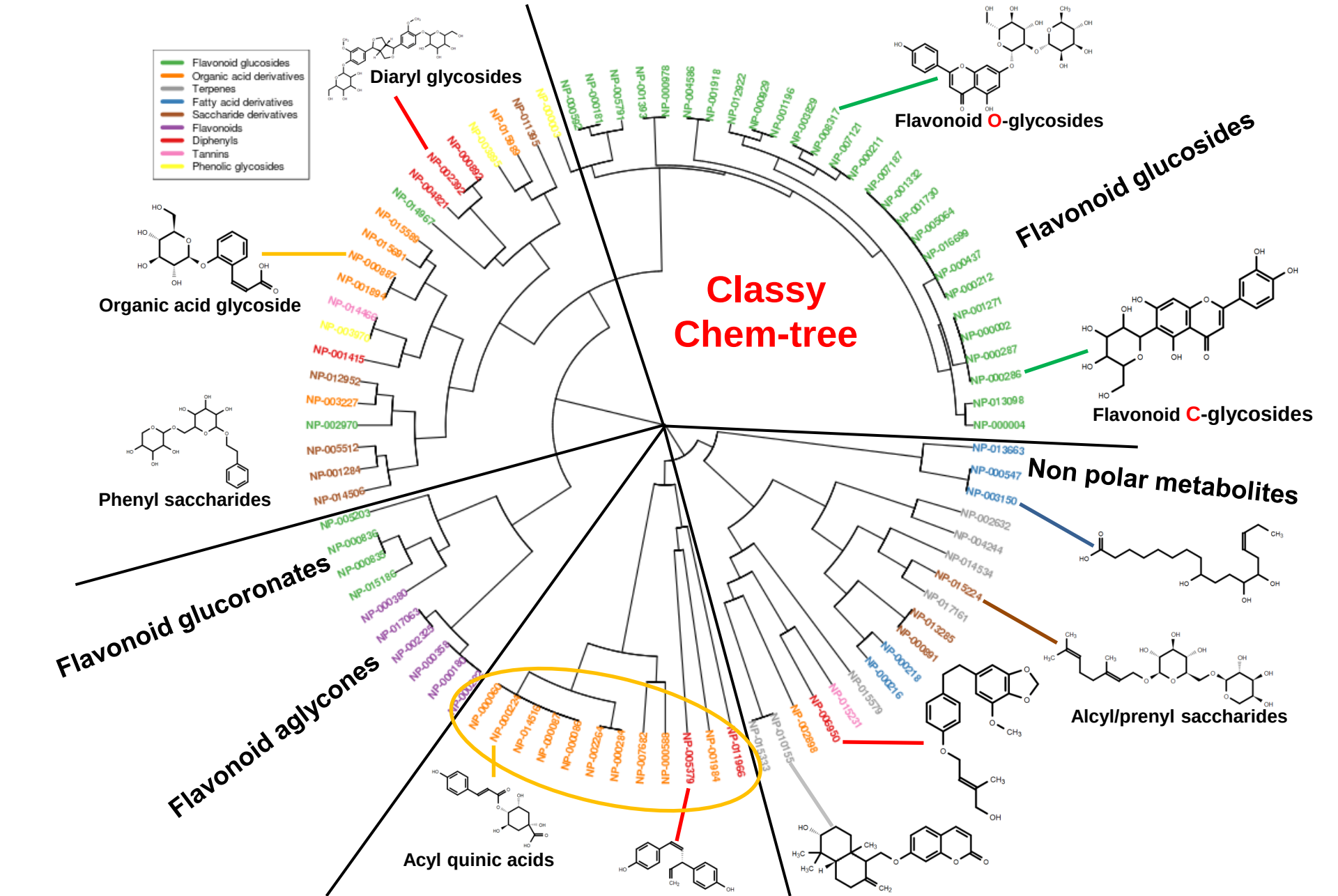
19 classes

Diverse, already compound specific

➢ **Manual refinement of 2nd level to 9 meaningful classes**

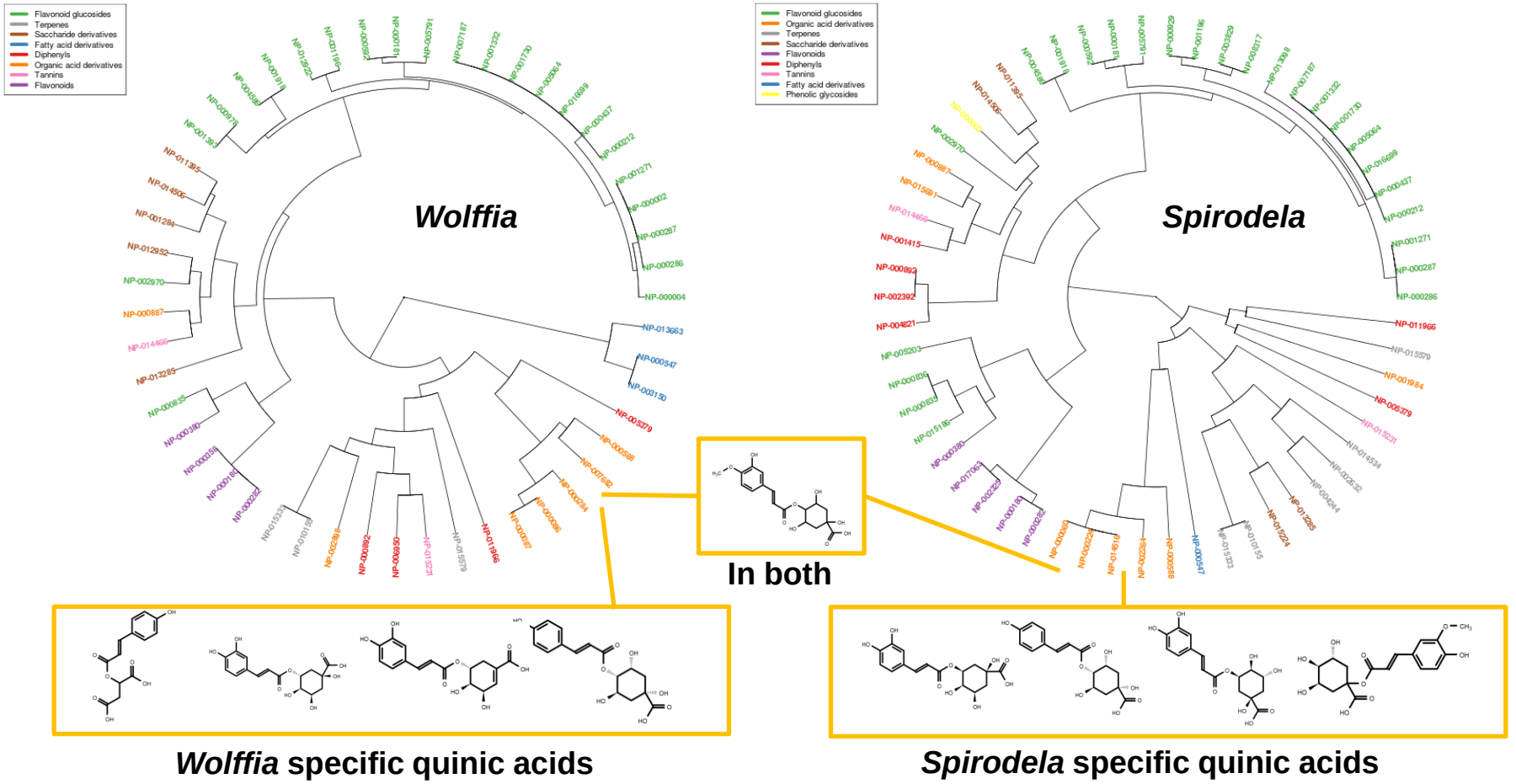
➢ **Example: Fatty acids and Glycerolipids (monoacyl, mainly FA)**


Flavonoid glucosides
Organic acid derivatives
Terpenes
Fatty acid derivatives
Saccharide derivatives
Flavonoids
Diphenyls
Tannins
Phenolic glycosides
Classy Chem-tree
Diaryl glycosides
Flavonoid O-glycosides
Flavonoid glucosides
Organic acid glycoside
Flavonoid C-glycosides
Non polar metabolites
Phenyl saccharides
Flavonoid glucoronates
Flavonoid aglycones
Alcyl/prenyl saccharides
Acyl quinic acids


Flavonoid glucosides
Organic acid derivatives
Terpenes
Fatty acid derivatives
Saccharide derivatives
Flavonoids
Diphenyls
Tannins
Phenolic glycosides
Classy Chem-tree
Diaryl glycosides
Flavonoid O-glycosides
Flavonoid glucosides
Organic acid glycoside
Flavonoid C-glycosides
Non polar metabolites
Phenyl saccharides
Flavonoid glucoronates
Flavonoid aglycones
Alcyl/prenyl saccharides
Acyl quinic acids

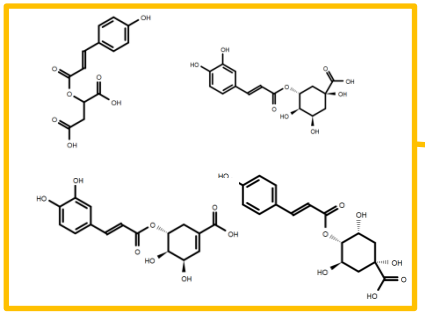
Collection screening – analysis of Lemna metabolites

➢Classy Chemtree presentation per species

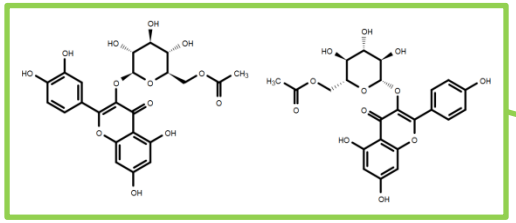


Collection screening – analysis of Lemna metabolites

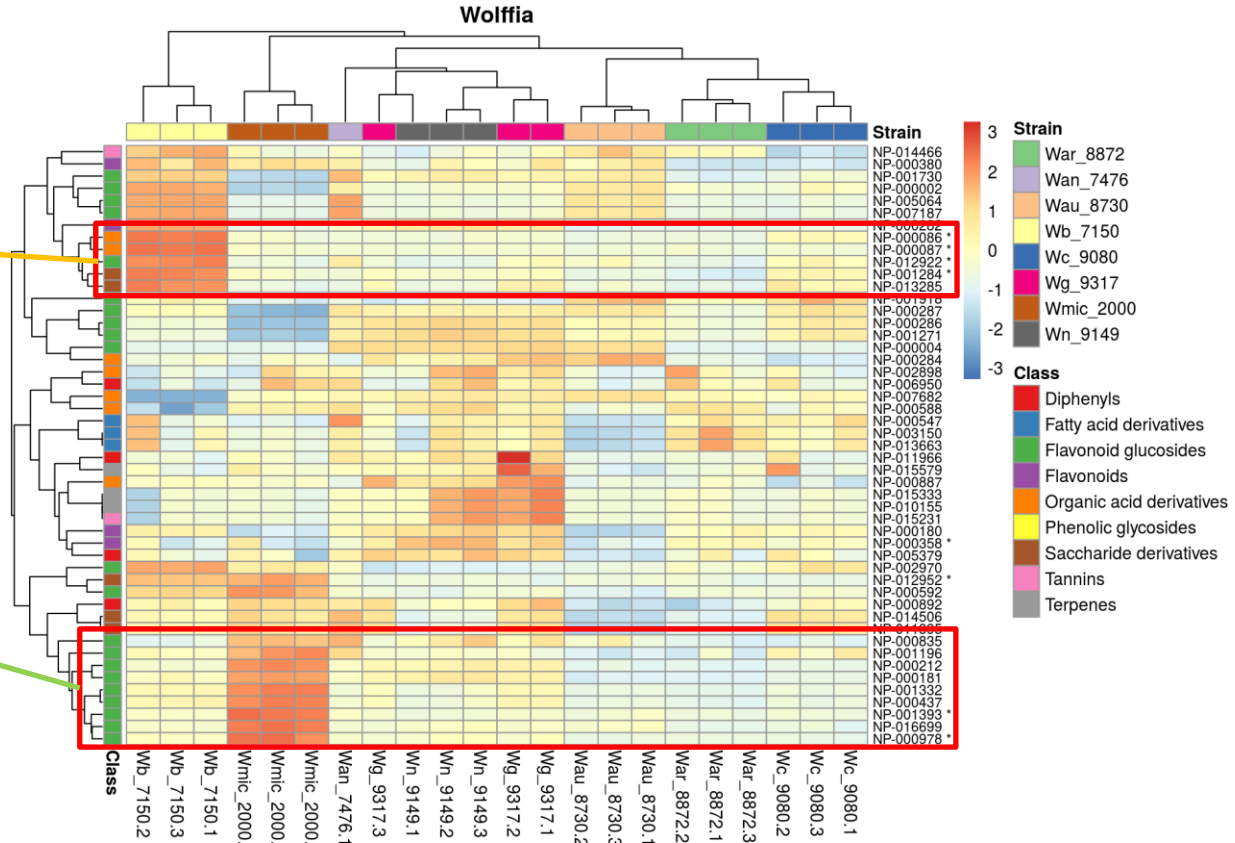
➢**Clustering analysis per genus combined with Classification**



Quinic acids



Acetylglucosyl Flavonoids



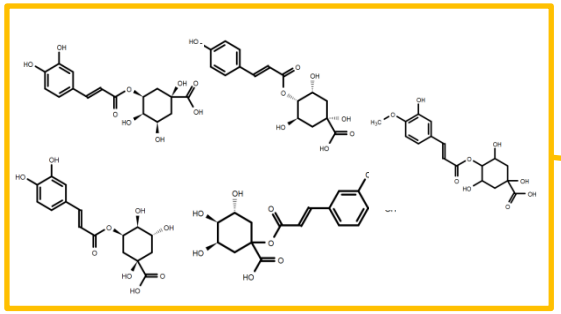
➢**Identification of high producers of *Wolffia* specialized metabolites**

WEIZMANN INSTITUTE OF SCIENCE

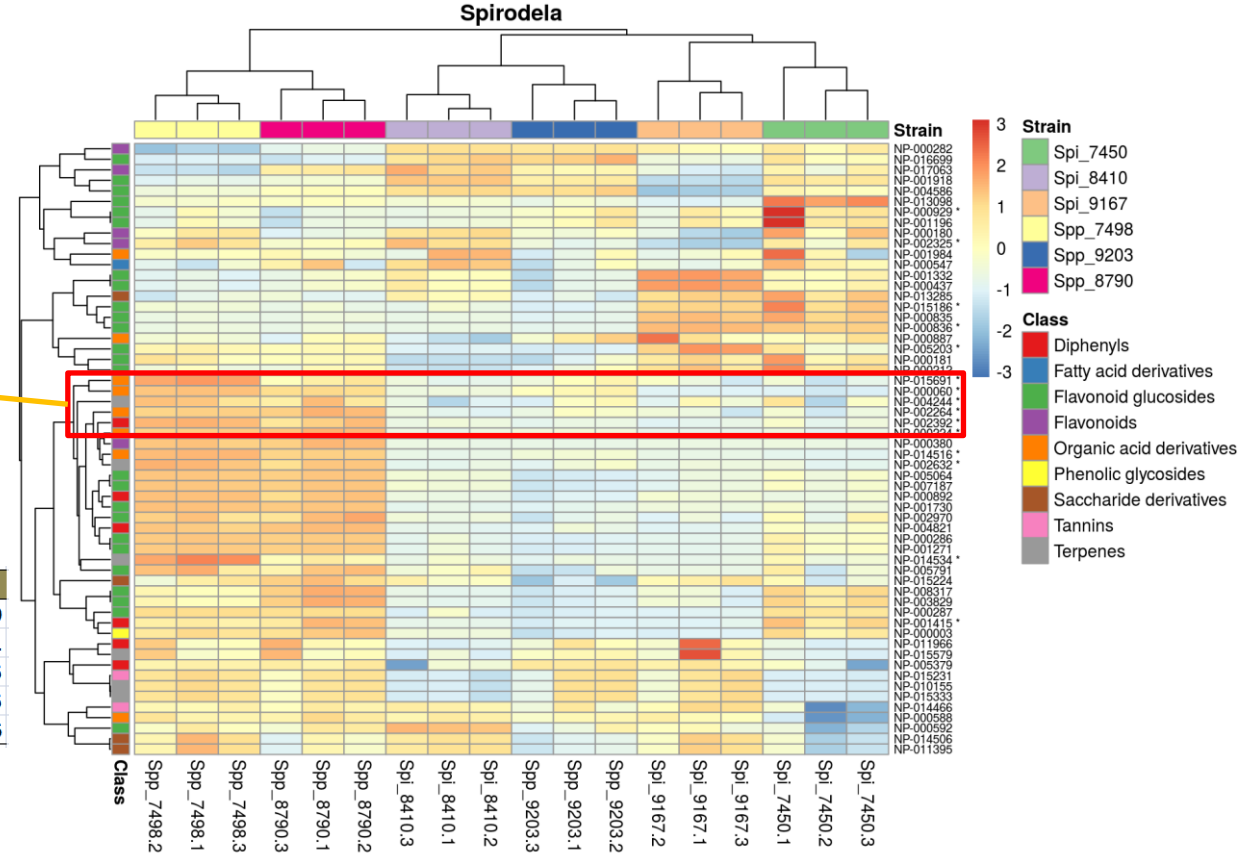
Collection screening – analysis of Lemna metabolites

➢ **Clustering analysis per genus combined with Classification**

Quinic acids



	Df	Sum Sq	Mean Sq	F value	Pr(>F)	FDR
NP-000060	5	16.8778	3.37555	331.387	2.01E-12	1.06E-10
NP-000224	5	16.9116	3.38233	459.282	2.87E-13	1.59E-11
NP-002264	5	15.8819	3.17638	34.0898	1.09E-06	3.05E-05
NP-014516	5	16.9807	3.39613	2108.62	3.16E-17	1.83E-15
NP-000588	5	15.0261	3.00522	18.2696	3.07E-05	0.000676



➢ ***Spirodela polyrhiza* 7498 (sequenced strain) is a producer of Quinic acids**

➢ **These acids are exclusively produced by *Spirodela* within the *Lemnaceae***

NCBI Resources How To Sign in to NCBI

PubMed.gov
US National Library of Medicine
National Institutes of Health

PubMed | Chlorogenic acid | Search

Create RSS Create alert Advanced Help

Article types
Clinical Trial
Review
Customize ...

Text availability
Abstract
Free full text
Full text

PubMed Commons
Reader comments
Trending articles

Publication dates
5 years
10 years
Custom range...

Species
Humans
Other Animals

Clear all

Show additional filters

Format: Summary **Sort by**: Most Recent **Per page**: 20 **Send to**

Filters: Manage Filters

Search Tip
Sort by **Best Match** to display results from highest to lowest relevance to your search terms.
Try it Now

Search results

Items: 1 to 20 of 3710

<< First < Prev Page 1 of 186 Next > Last >>

1. Blanching influences the phenolics composition, antioxidant activity, and inhibitory effect of *Adansonia digitata* leaves extract on α-amylase, α-glucosidase, and aldose reductase.
Irondi EA, Akintunde JK, Agboola SO, Boligon AA, Athayde ML.
Food Sci Nutr. 2016 May 25;5(2):233-242. doi: 10.1002/fsn3.386.
PMID: 28265358 **Free Article**
Similar articles

2. Influence of Indole-3-Acetic **Acid** and Gibberellic **Acid** on Phenylpropanoid Accumulation in Common Buckwheat (Fagopyrum esculentum Moench) Sprouts.
Park CH, Yeo HJ, Park YJ, Morgan AM, Valan Arasu M, Al-Dhabi NA, Park SU.
Molecules. 2017 Feb 28;22(3). pii: E374. doi: 10.3390/molecules22030374.
PMID: 28264513 **Free Article**
Similar articles

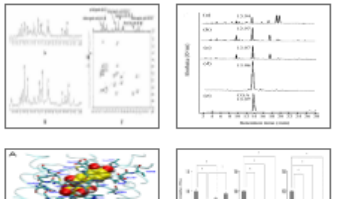
3. Comparative pharmacokinetics of **chlorogenic acid** in beagles after oral administrations of single compound, the extracts of Lonicera japanica, and the mixture of **chlorogenic acid**, baicalin, and Forsythia suspense.
Liu R, Lai K, Xiao Y, Ren J.
Pharm Biol. 2017 Dec;55(1):1234-1238. doi: 10.1080/13880209.2017.1296002.

Results by year

Download CSV

Related searches
chlorogenic acid weight loss
chlorogenic acid coffee
chlorogenic acid review
chlorogenic acid cancer
chlorogenic acid antioxidant

PMC Images search for *Chlorogenic acid*



WEIZMANN INSTITUTE OF SCIENCE

Lemna collection screening summary & perspectives

- High confidence identification of 88 (+15) secondary metabolites in *Lemna*
- Deep analysis of MatchWeiz validation
- Integration of semi-automated compound classification into chemtree and clustering visualisation tool for metabolomics
- Identification of *Wolffia & Spirodela* specific metabolites as Acyl quinic acids

- Improvement of MatchWeiz module (RT, output filtration, derivative search)
- Reduction of necessary validation spiking experiments
- Integration of results in Metabolic network of *Lemna* (SpirodelaCyc)
- Pathway elucidation, production of valuable metabolites

Overview

Part I – metabolic profiling of *Lemnaceae*

Part II – DLEMMA for metabolite identification

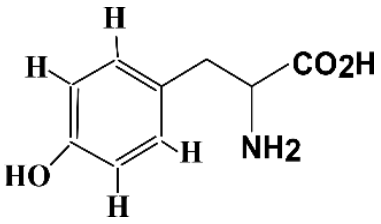
Part III – flux analysis using *Spirodela polyrhiza*

Combining Dual Isotope Labeling of Metabolites for Metabolome Analysis (DLEMMA) with MALDI-MSI

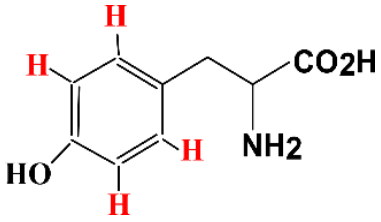
- Difficulties of metabolite identification in MSI and metabolomics
- DLEMMA helps in structure elucidation according to their labeling patterns
- DLEMMA enables tracking a particular metabolic pathway by enriching in this pathway with precursor feeding

1. Experimental Design

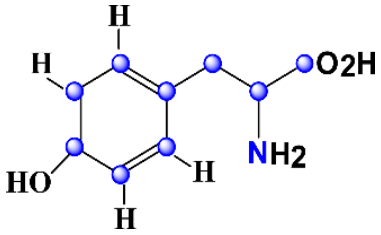
un-labelled tyrosine



labelled I



labelled II



A: control

B: un-labelled

C: labelled I

D: labelled II

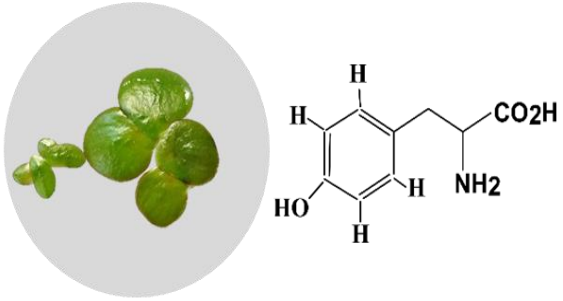
E: labelled I + II

1. Experimental Design

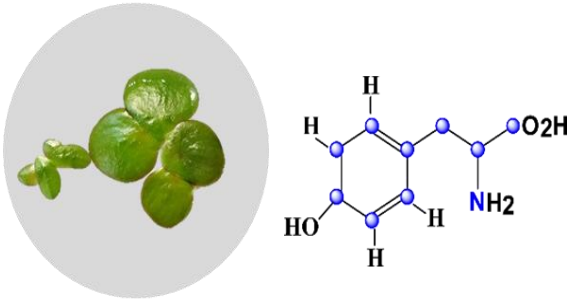
1. Why feed *Lemna* separately? Can we feed all the 3 tyrosines in one *Lemna* group?

Suppose:

- Analyte X in Group. B, *m/z* 142.0817, RT=5.52 min
- Analyte Y in Group. D, m/z 151.1119 (=142.0871+9 C^{13}), RT= 5.52 min



B: un-labelled tyrosine



D: labelled II

Is analyte Y the 9 ^{13}C labelled X?

- No. Unless Y is only detected in Group. D and E.
- The experiment design efficiently removes false positives

1. Experimental Design

2. Why do we need the control group A?

- We are creating stress to plants by feeding them
- Some metabolites are produced upon stress
- Group. A helps to remove those stress-induced-metabolites

A: control

2. Workflow

1. Sample Extraction

↓

2. LC-MS and LC-MS/MS

- positive and negative

↓

3. Data preprocessing

- peak-picking with XCMS
- Isotope filtering (home-written R script)

↓

4. Metabolite identification & network construction

- exact m/z, RT, and MS/MS fragmentation
- labeling pattern and database search

- Auto isotope labeling search according to the experiment design

Exp. B		**EXP. C**			**EXP. D**		
m/z	RT	*m/z*	RT	label	*m/z*	RT	label
105.0701	3.05	108.0865	3.08	3	113.1197	3.07	8
107.0495	2.16	111.0755	2.09	4	113.0879	2.06	6
120.0805	4.88	124.1059	4.85	4	128.1309	4.88	8
…	…	…	…	…	…	…	…

- Reduce from 20,000 to ~ 100 analytes

3. Example of Analyte Identification

Exp. B		EXP. C			EXP. D		
m/z	RT	*m/z*	RT	label	*m/z*	RT	label
267.1345	2.75	271.1599	2.74	4	276.1579	2.74	9

3. Example of Analyte Identification

Exp. B		EXP. C			EXP. D		
m/z	**RT**	*m/z*	RT	label	*m/z*	RT	label
267.1345	**2.75**	271.1599	2.74	4	276.1579	2.74	9

$C_{13}H_{18}N_2O_4$

3. Example of Analyte Identification

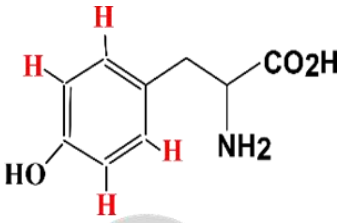
Exp. B		EXP. C			EXP. D		
m/z	**RT**	*m/z*	RT	label	*m/z*	RT	label
267.1345	**2.75**	271.1599	2.74	4	276.1579	2.74	9

$C_{13}H_{18}N_2O_4$: with this formula > 14,000 compounds found in Scifinder

3. Example of Analyte Identification

Exp. B		EXP. C			EXP. D		
m/z	RT	***m/z***	**RT**	**label**	*m/z*	RT	label
267.1345	2.75	**271.1599**	**2.74**	**4**	276.1579	2.74	9

$C_{13}H_{18}N_2O_4$: with this formula > 14,000 compounds found in Scifinder



EXP. C: labelled I

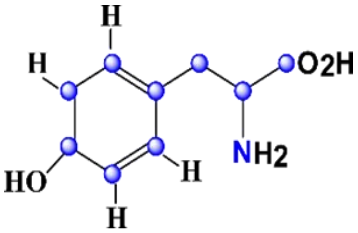
there must be one aromatic ring in this compound, and with 4 H on it, 313 compounds

WEIZMANN INSTITUTE OF SCIENCE

3. Example of Analyte Identification

Exp. B		EXP. C			EXP. D		
m/z	RT	*m/z*	RT	label	***m/z***	**RT**	**label**
267.1345	2.75	271.1599	2.74	4	**276.1579**	**2.74**	**9**

$C_{13}H_{18}N_2O_4$: with this formula > 14,000 compounds found in Scifinder



EXP.D: labelled II

there must be one aromatic ring, at least 8 C bone, on it, 403 compounds

3. Example of Analyte Identification

Exp. B		EXP. C			EXP. D		
m/z	RT	***m/z***	**RT**	**label**	***m/z***	**RT**	**label**
267.1345	2.75	**271.1599**	**2.74**	**4**	**276.1579**	**2.74**	**9**

$C_{13}H_{18}N_2O_4$: with this formula > 14,000 compounds found in Scifinder

313 in C — **403 in D**

48 in C and D

3. Example of Analyte Identification

Exp. B		EXP. C			EXP. D		
m/z	RT	***m/z***	**RT**	**label**	***m/z***	**RT**	**label**
267.1345	2.75	**271.1599**	**2.74**	**4**	**276.1579**	**2.74**	**9**

$C_{13}H_{18}N_2O_4$: with this formula > 14,000 compounds found in Scifinder

313 in C — **403 in D**

48 in C and D → **4 are biologically reported**

3. Example of Analyte Identification

Exp. B		EXP. C			EXP. D		
m/z	RT	***m/z***	**RT**	**label**	***m/z***	**RT**	**label**
267.1345	2.75	**271.1599**	**2.74**	**4**	**276.1579**	**2.74**	**9**

$C_{13}H_{18}N_2O_4$: with this formula > 14,000 compounds found in Scifinder

313 in C **403 in D**

48 in C and D → **4 are biologically reported**

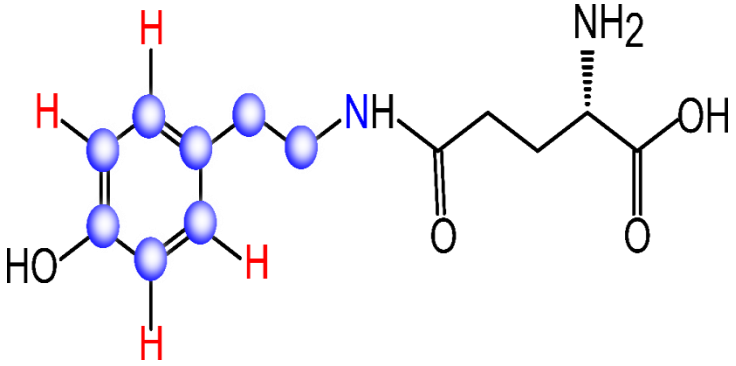
↓

1 hit with our MS/MS data

3. Example of Analyte Identification

Exp. B		EXP. C			EXP. D		
m/z	RT	*m/z*	RT	label	*m/z*	RT	label
267.1345	2.75	271.1599	2.74	4	276.1579	2.74	9

Glutamine side chain $C_{13}H_{18}N_2O_4$



4. When MALDI Imaging Comes…

What can they do to each other?

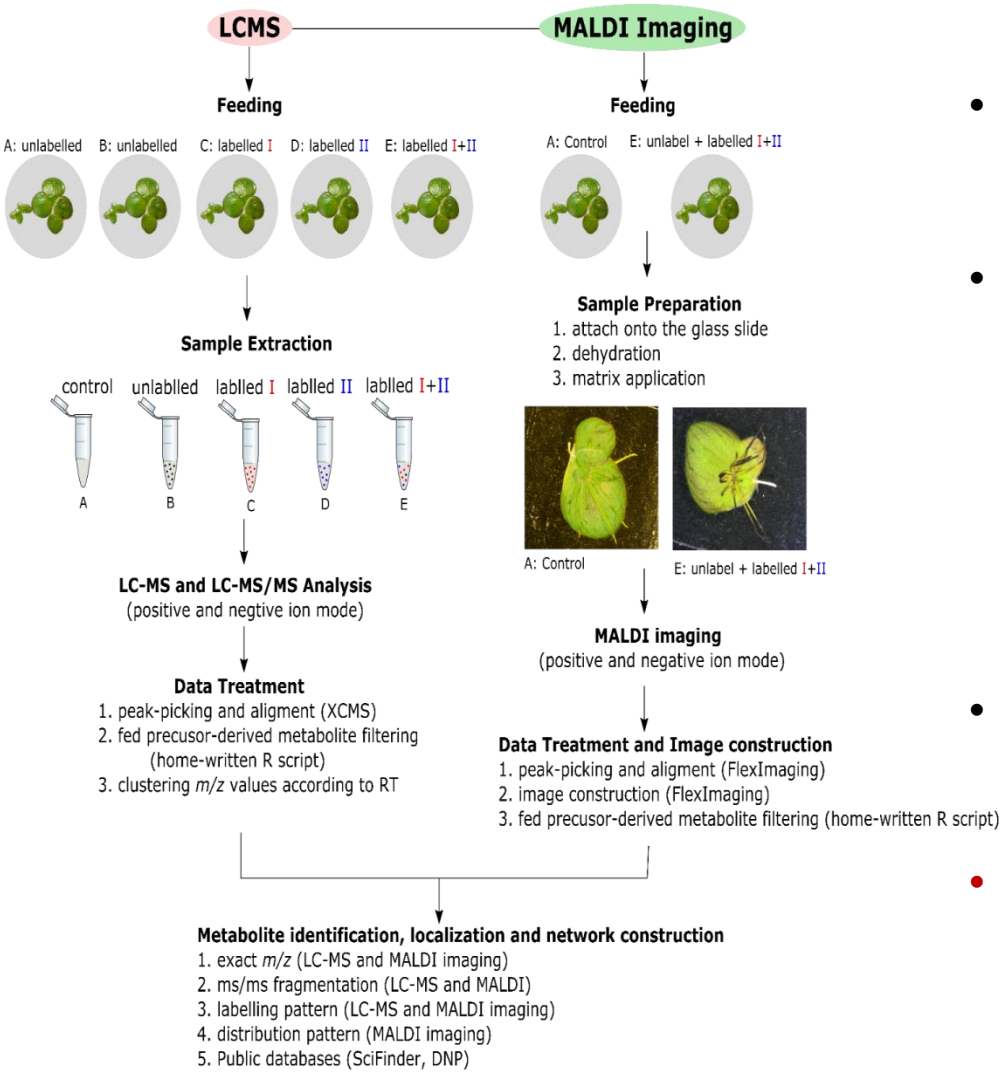
DLEMMA to MALDI MSI:

- Help in metabolite identification
- Increase the analyte concentrations by precursor feeding, favoring their detection by MALDI imaging

MALDI MSI to DLEMMA:

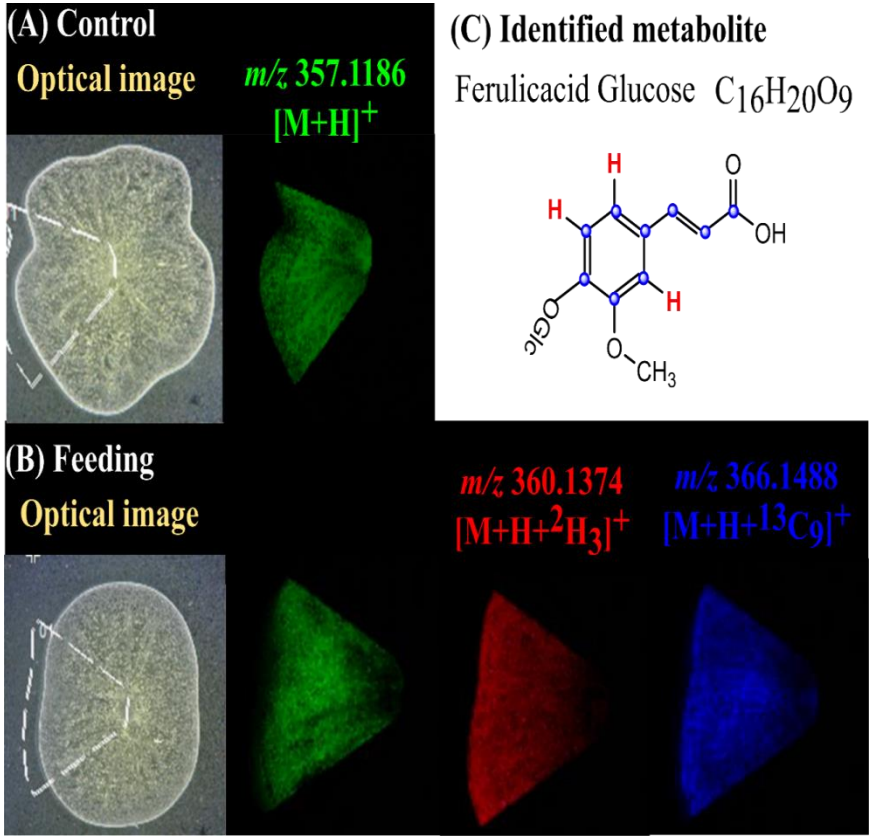
- Enhance metabolite identification capability (unlablled and labelled analytes should have the same distribution pattern)
- Provide information regarding metabolite distribution

5. DLEMMA-MALDI MSI Experiment Design

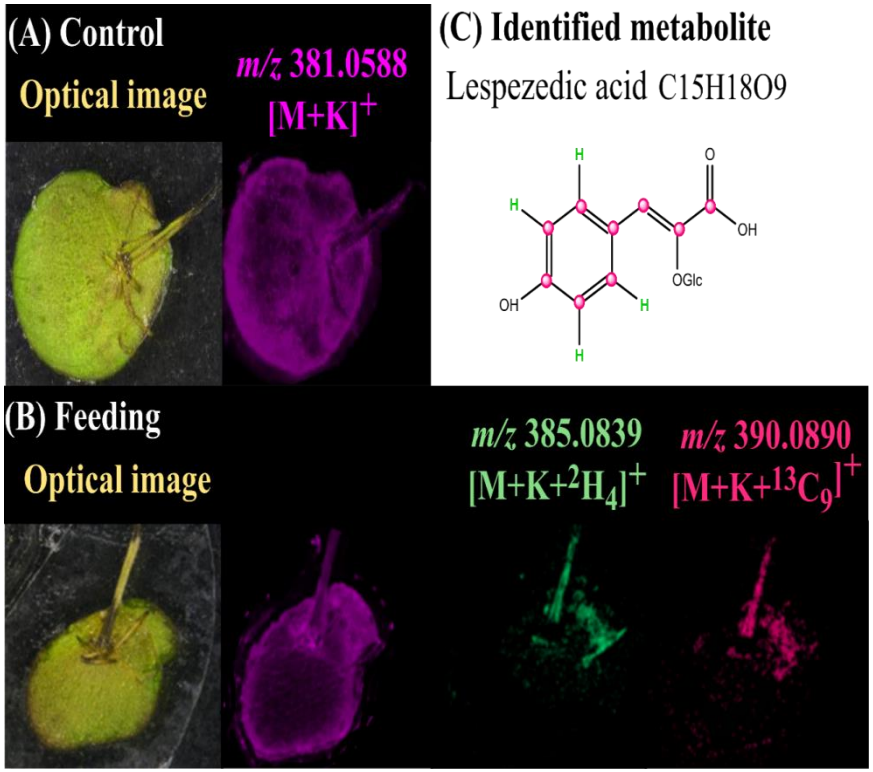


- The workflow is similar as conventional metabolomics analysis
- In MALDI MSI, un-labelled precursor and its two isotopes were added to the same tissue because we want to compare their distribution
- *Lemna* and tomato fruits were used in this study
- Tyrosine, phenylalanine and tryptophan as three precursors

Example 1. Un-labelled and Labelled analytes show the same distribution

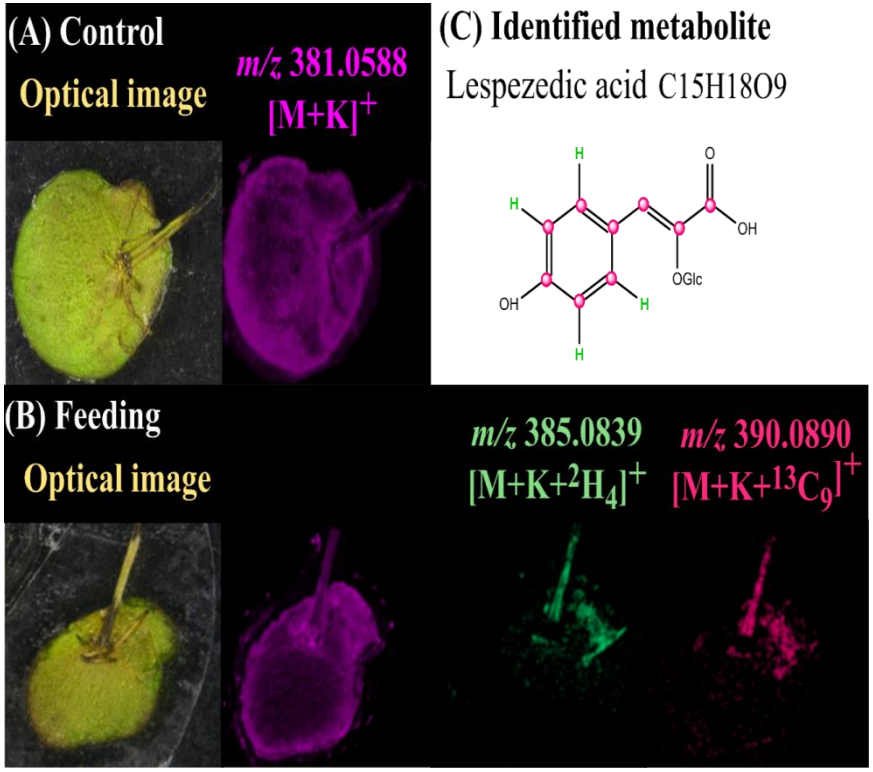


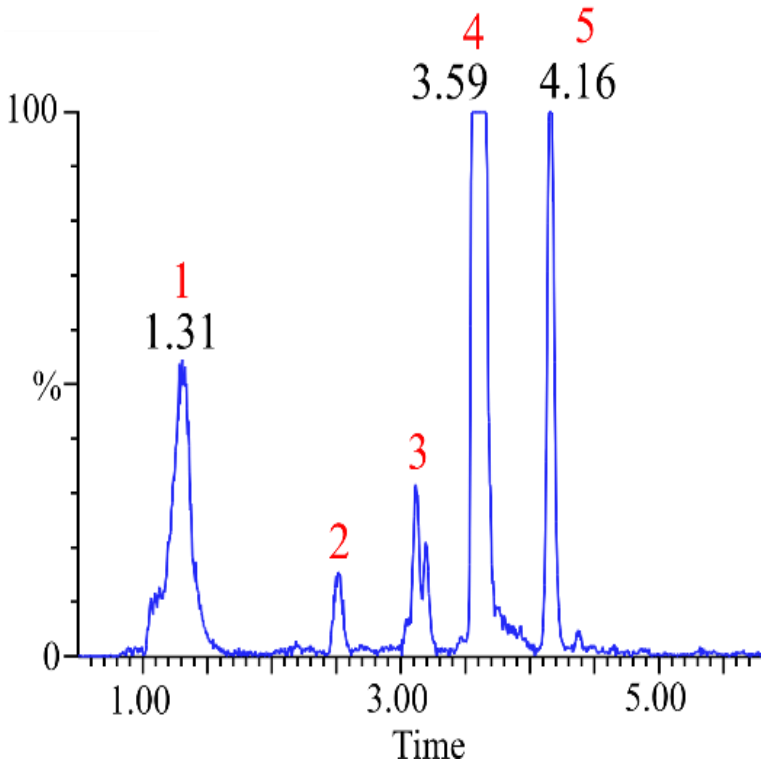
Example 2. Un-labelled and Labelled analytes show different distribution



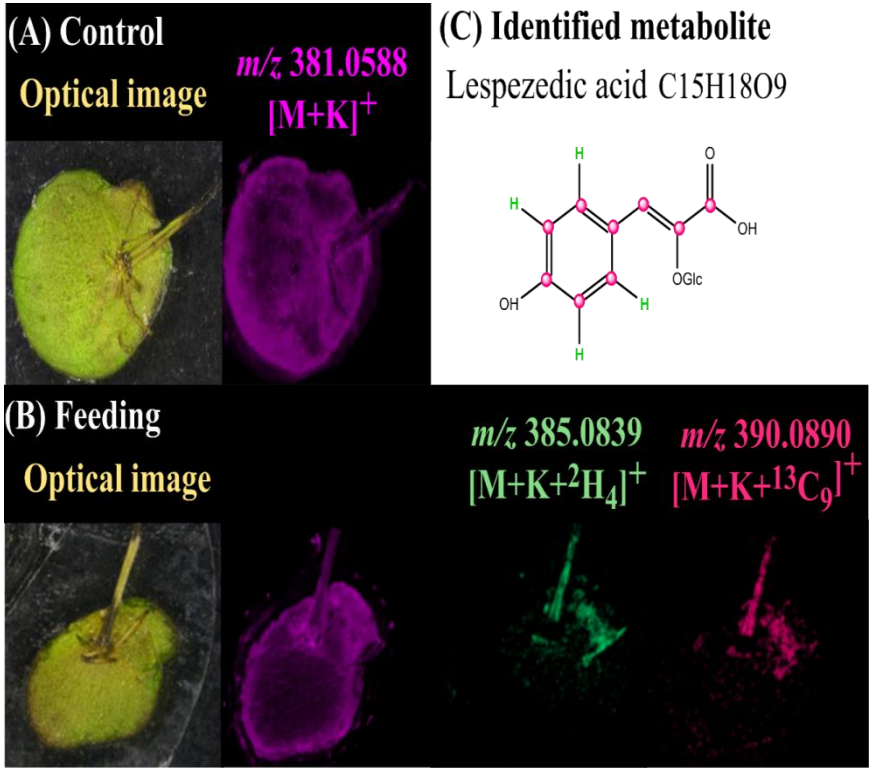
WEIZMANN INSTITUTE OF SCIENCE

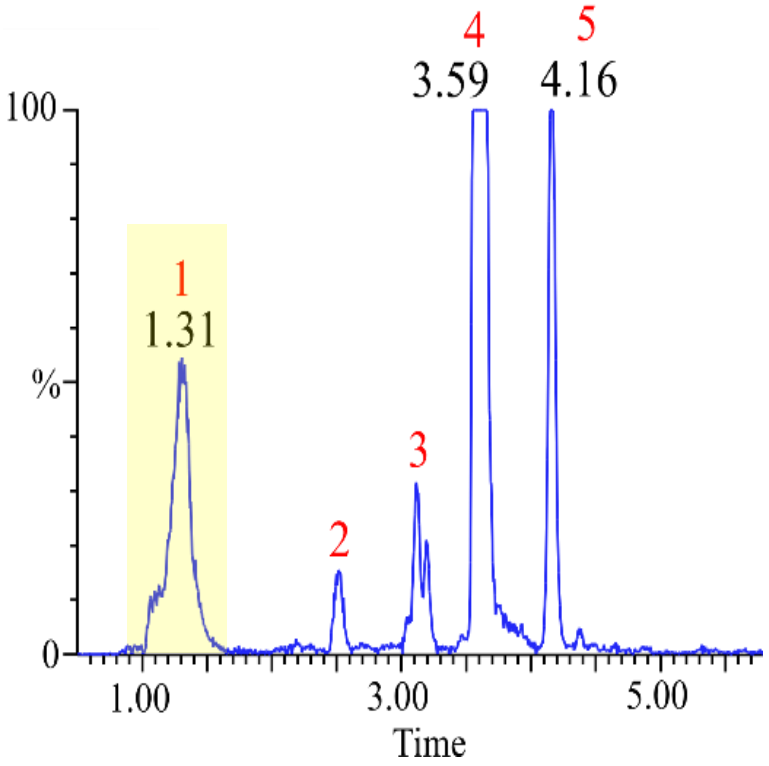
Example 2. Five isomers are Found





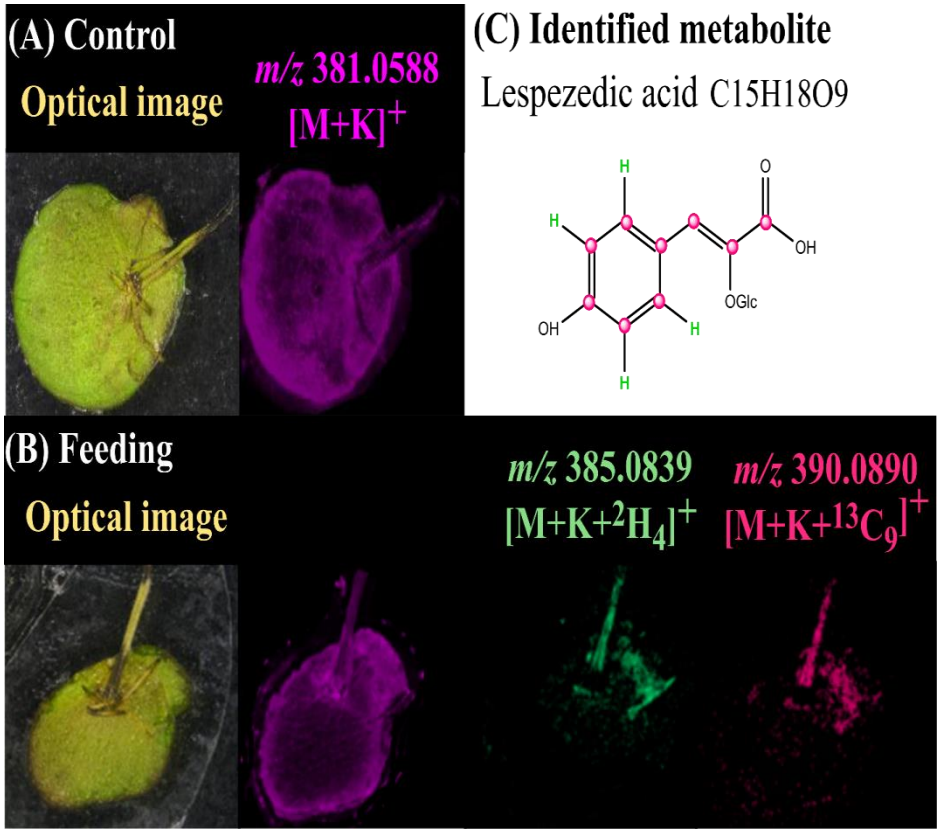
Example 2. Only One of Them is Labelled





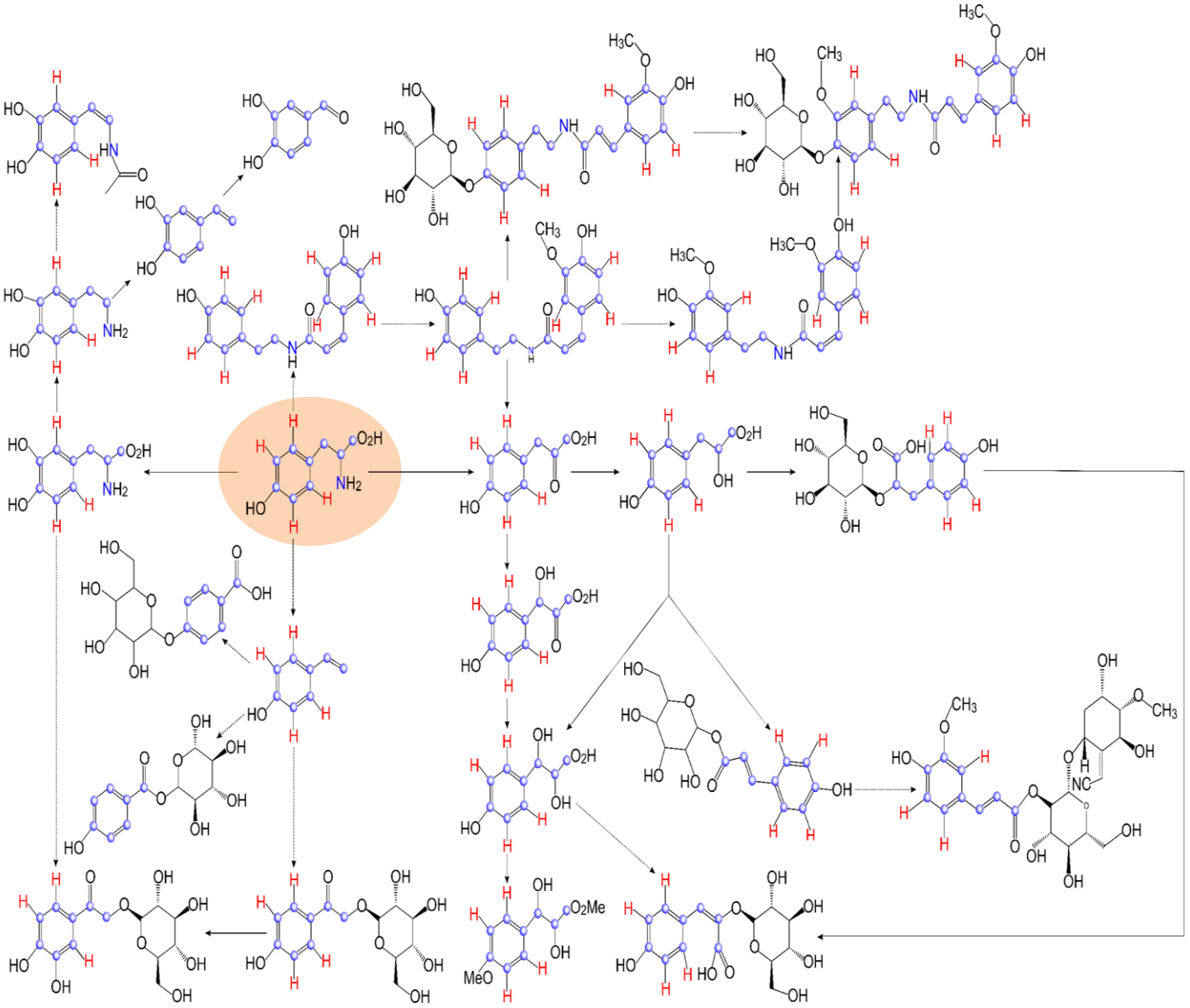
WEIZMANN INSTITUTE OF SCIENCE

Example 2. Only One of Them is Labelled



- MSI is unable to distinguish isomers
- Isomers might have different metabolic pathways
- DLEMMA helps to differentiate them and represents the true distribution of our analytes of interest

Metabolic Network with Identified Metabolites



Conclusion

DLEMMA-MALDI MSI:

- **Helps in metabolite identification**
- **Allows metabolic network construction**
- **Provide spatial information**

In particular:

- **DLEMMA helps to eliminate the artifacts in MALDI imaging**

Overview

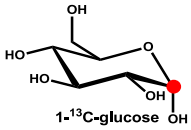
Part I – metabolic profiling of *Lemnaceae*

Part II – DLEMMA for metabolite identification

Part III – flux analysis using *Spirodela polyrhiza*

^{13}C metabolic flux analysis using Spirodela

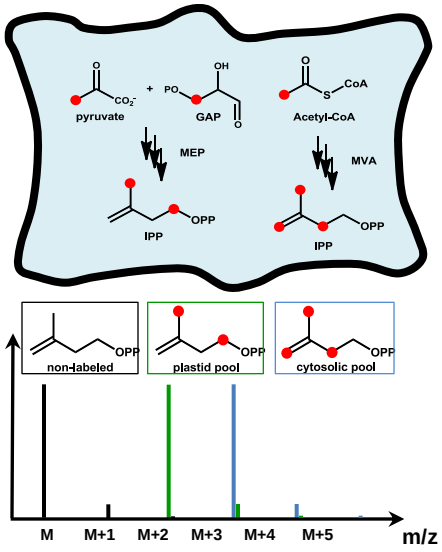
^{13}C-MFA



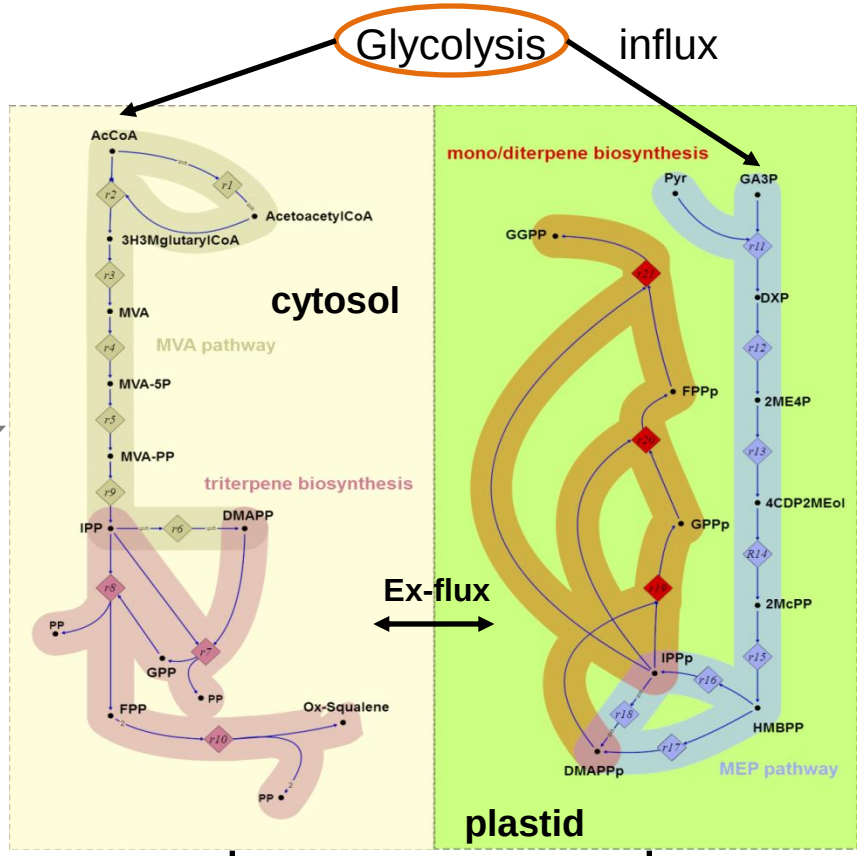
Spirodela polyrrhiza

$^{13}CO_2$

Isotopomer pattern



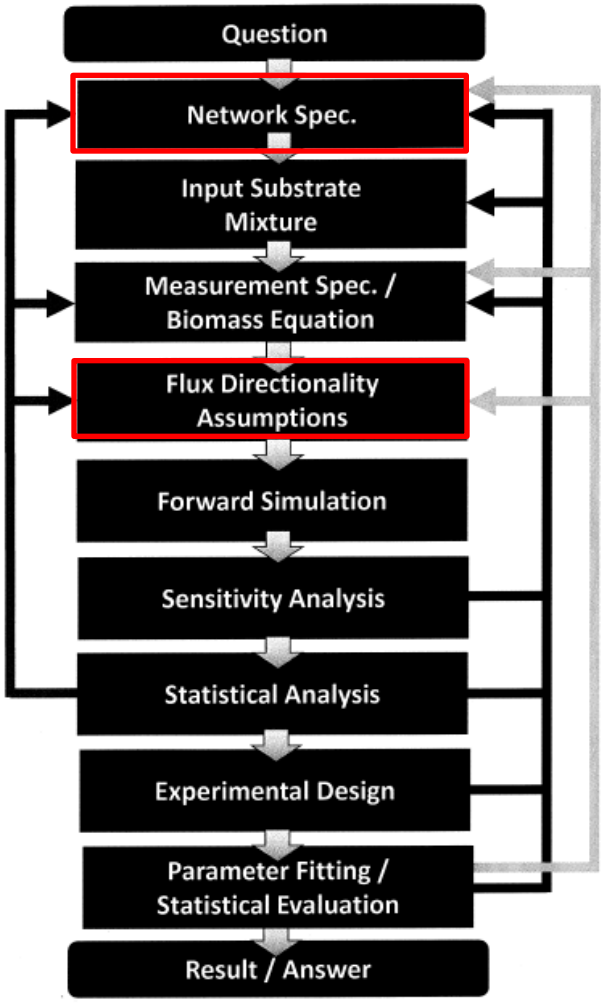
Flux map



Triterpenes, Sterols (efflux)

Mono-, di-, tetra-terpene, (efflux)

Flux analysis – network construction

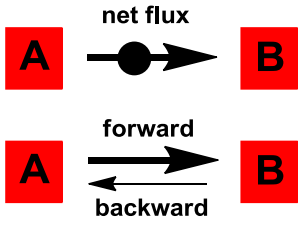


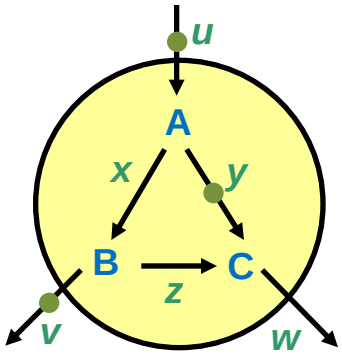
→Stoichiometry of the network (DOF)

→closed carbon balance

→influx and efflux

→network structure (directionality, cycles,…)





A: u=x+y
B: x=v+z
C: w=y+z

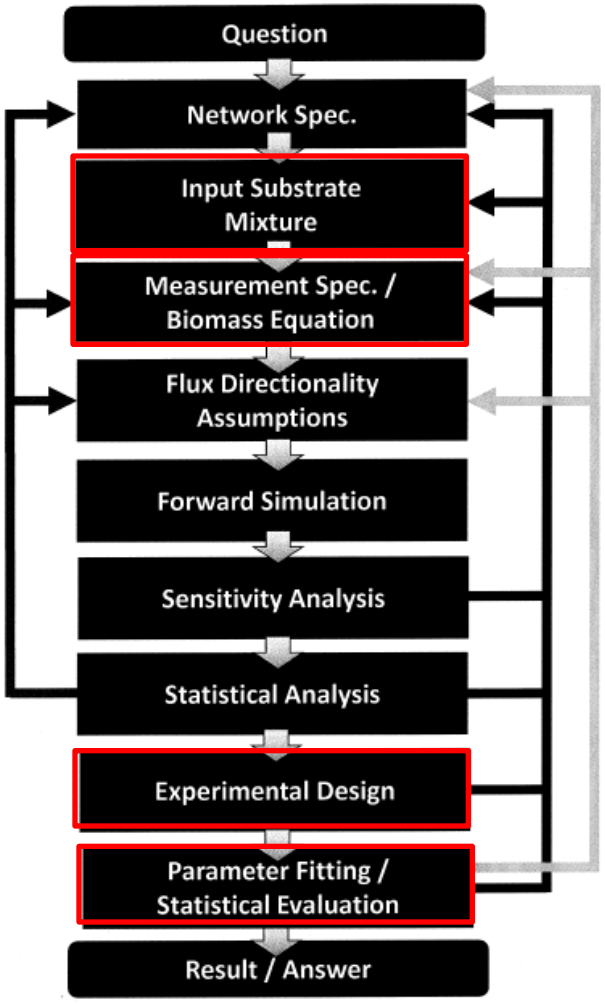
→ free fluxes

→constraints

→Example: *E. coli CCM model:*

59 metabolites, 68 reactions

Flux analysis – experimental considerations



→labeled carbon source (1-^{13}C-Glu, CO_2)

→analytical technique (MS, NMR)

→atom transitions (where does my label go?)

GA3P	**+**	**S7P**	**>**	**E4P**	**+**	**F6P**
#ABC	**+**	**#abcdefg**	**>**	**#defg**	**+**	**#abcABC**

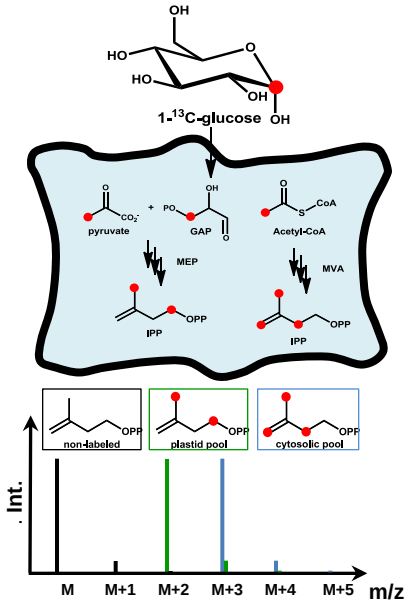
→number of measurements

→what experiments?

→compounds to measure

→experimental errors

→statistical analysis

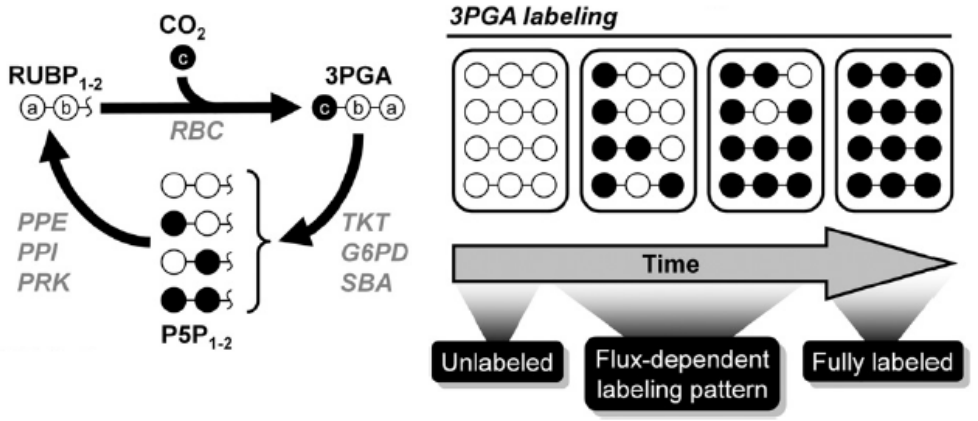


Flux analysis – in whole plants, the major challenges

→**Definition of "metabolic steady state"**

→**compartmentation :** parallel pathways in different compartments act differently

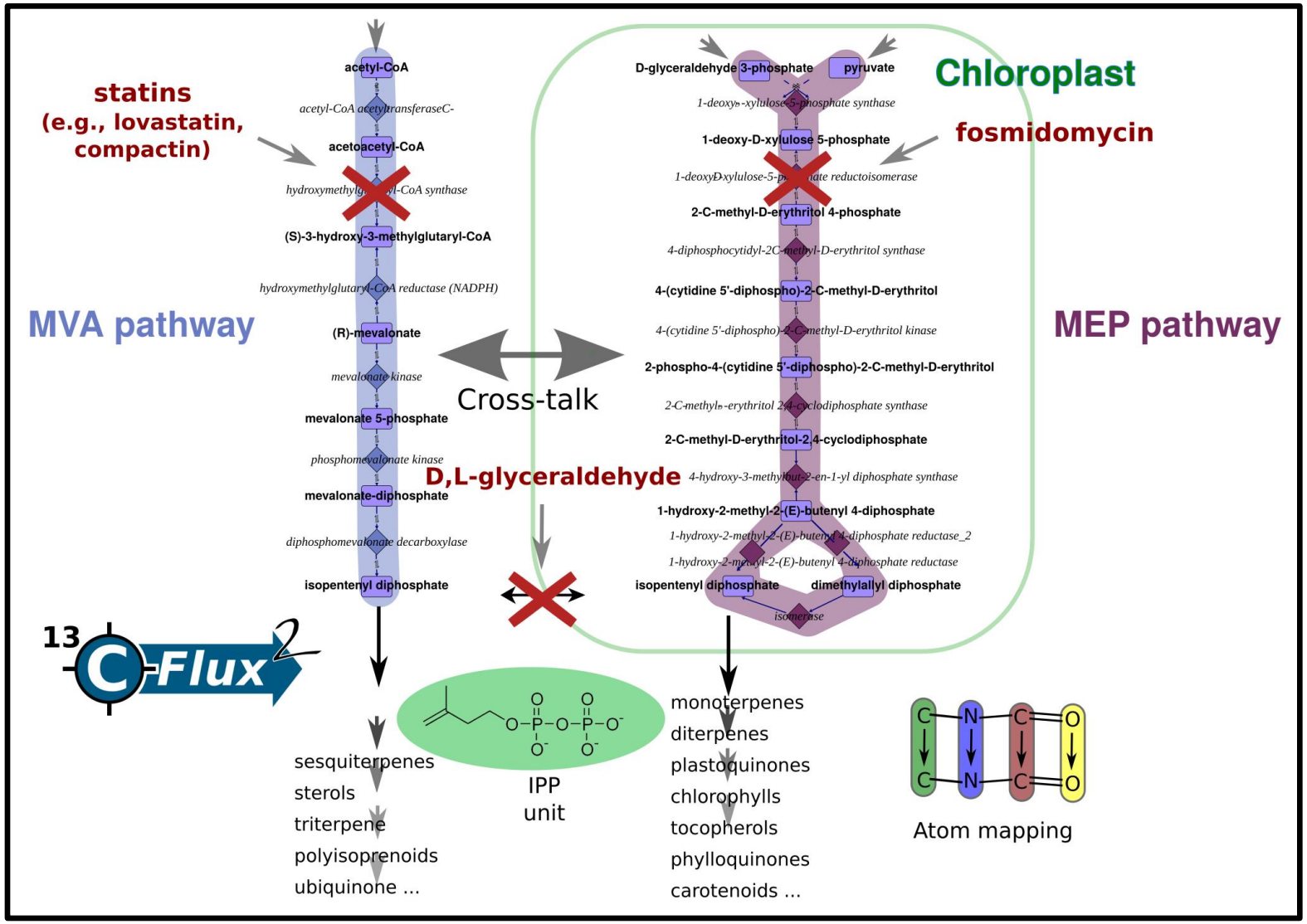
→**phototrophic conditions :** $^{13}CO_2$ as a carbon source results in loss of flux information at "isotopic steady state"



Young et al. 2011

→ **Is "steady-state" MFA possible for plants ???**

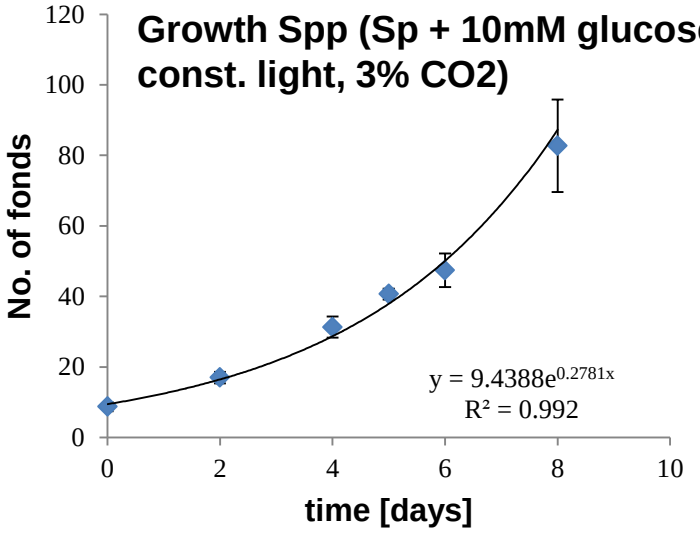
Flux network (Nadine)



WEIZMANN INSTITUTE OF SCIENCE

Metabolic steady state – MEP+MVA pathway

- time-course experiment and relative quantification of relevant intermediates
- linear pathways without branching, in theory analysis of early and late intermediates should be informative enough



- analysis of MEP and MVA intermediates and terpene end products
- method development for pathway intermediates

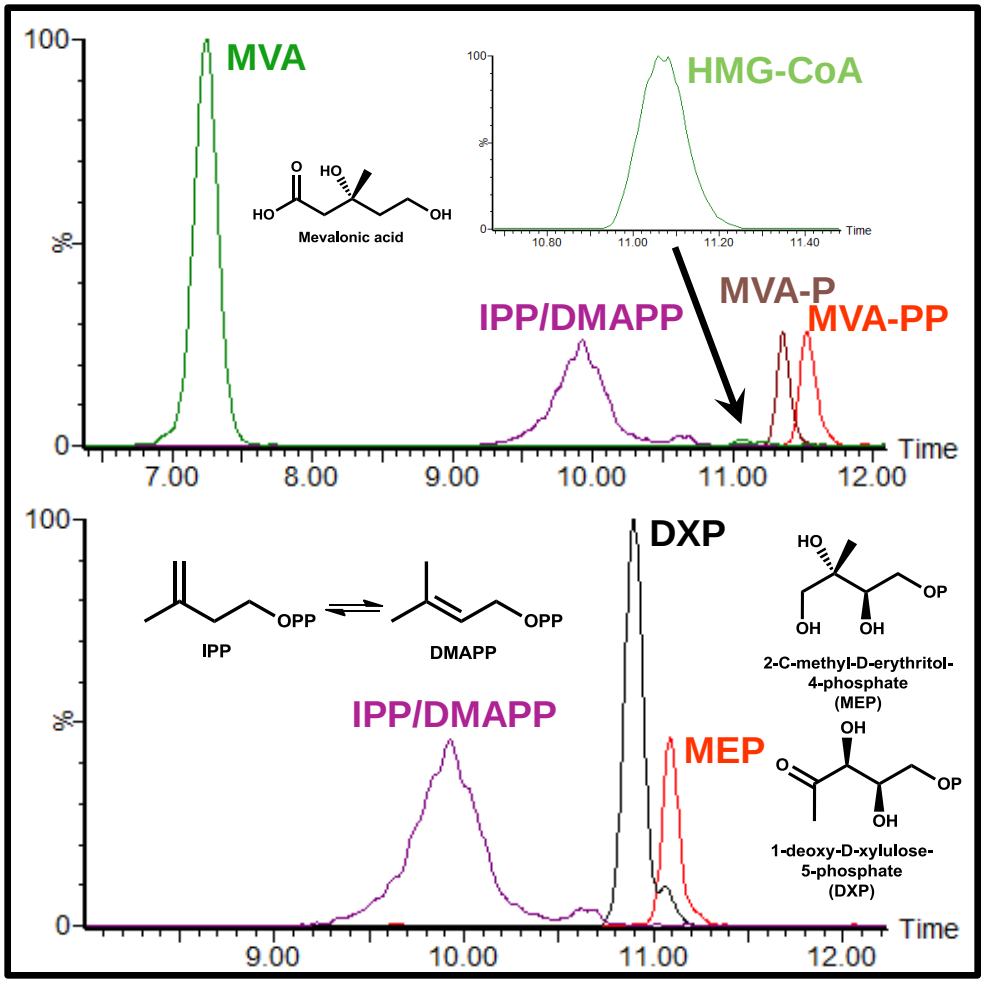
WEIZMANN INSTITUTE OF SCIENCE

Metabolic steady state – MEP+MVA pathway

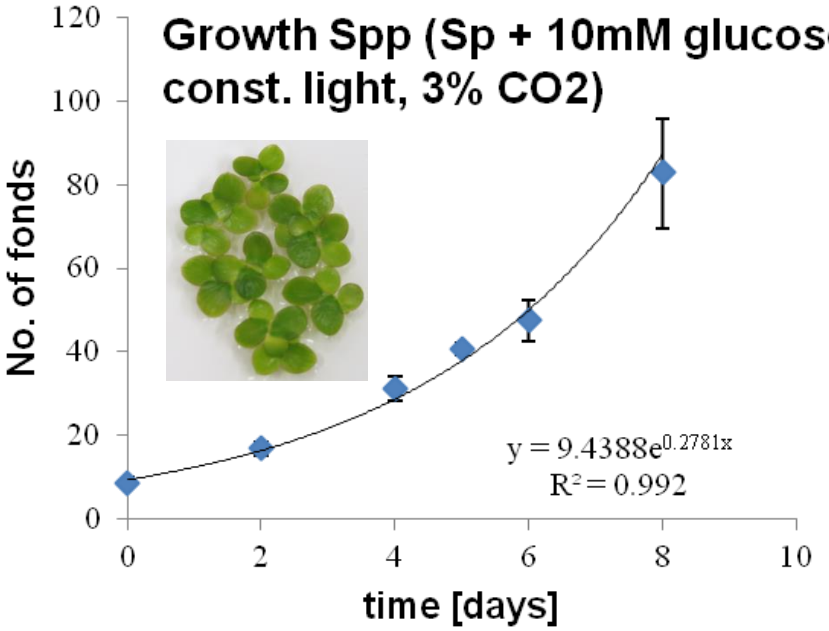
➢**LC-TQ MS method** (Zic-pHILIC-TQ-MS (MRM) (gradient elution, A: 50mM NH_4OAc pH 10 in 3%ACN, B: ACN)

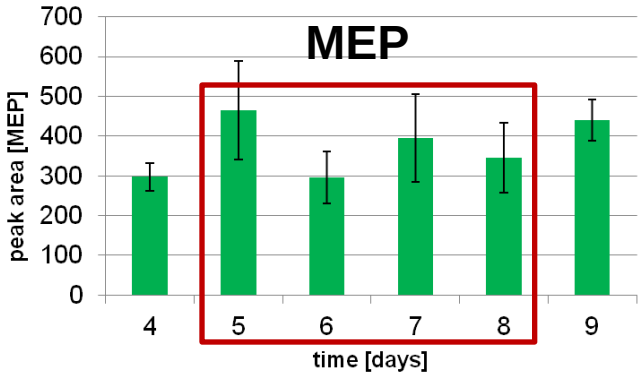
(Li et al. 2013)

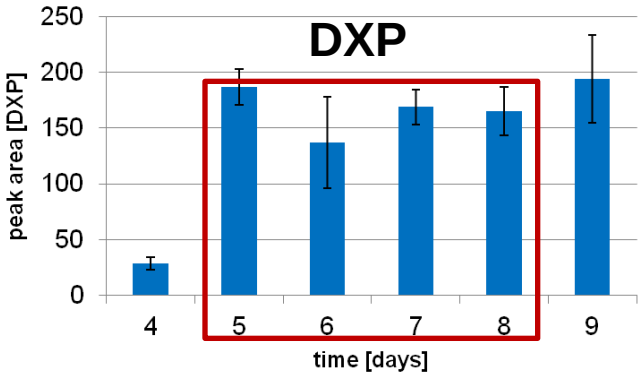
compound	transition	CE [eV]	RT [min]
MVA	147.2 --> 59	23	7.22
MVA-P	227.1 --> 97.1	14	11.35
MVA-PP	307.1 --> 79	25	11.55
HMG-CoA	454.6 --> 686.3	17	11.06
DXP	213.05 --> 97	13	10.88
MEP	215.2 --> 79	20	11.1
IPP/DMAPP	245.1 --> 79	20	9.9

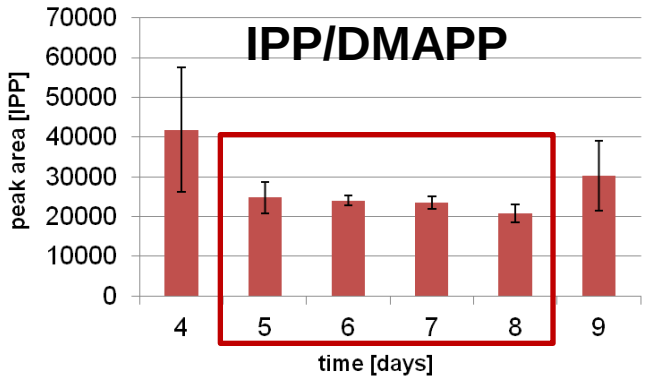


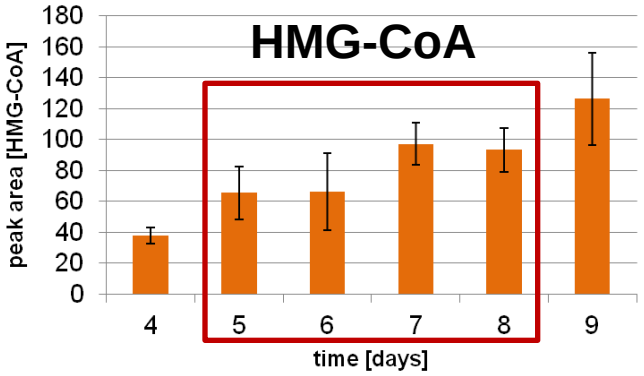
Metabolic steady state – MEP+MVA pathway



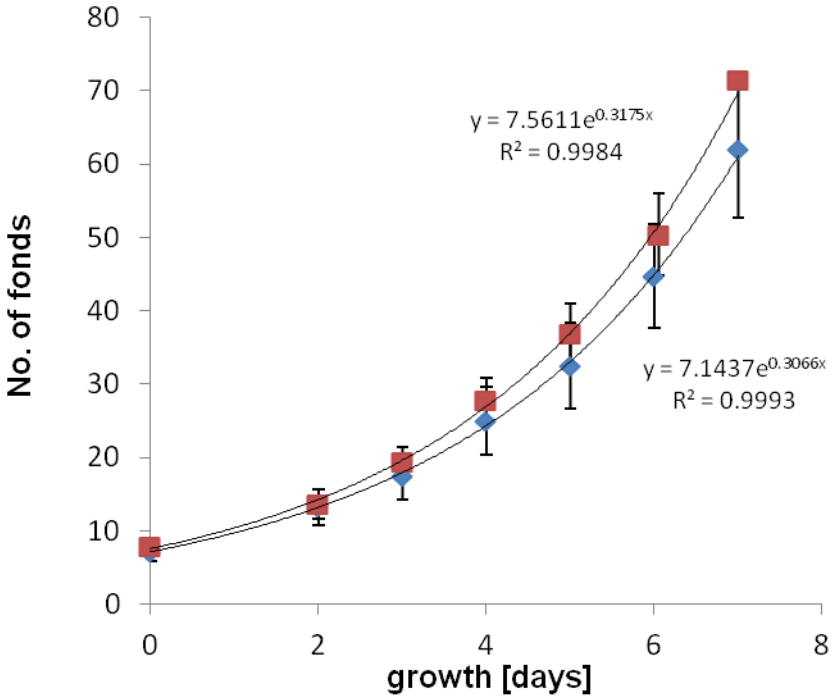








Metabolic steady state – MEP+MVA pathway

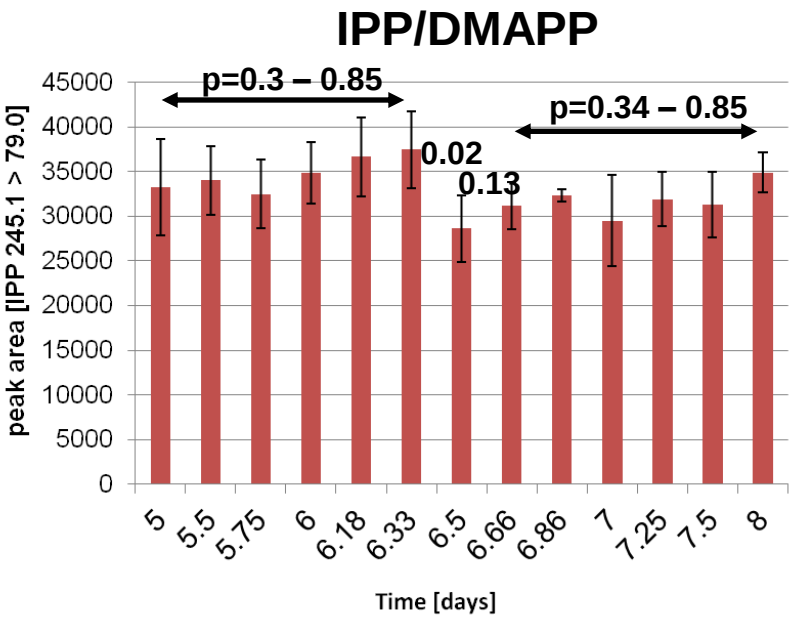


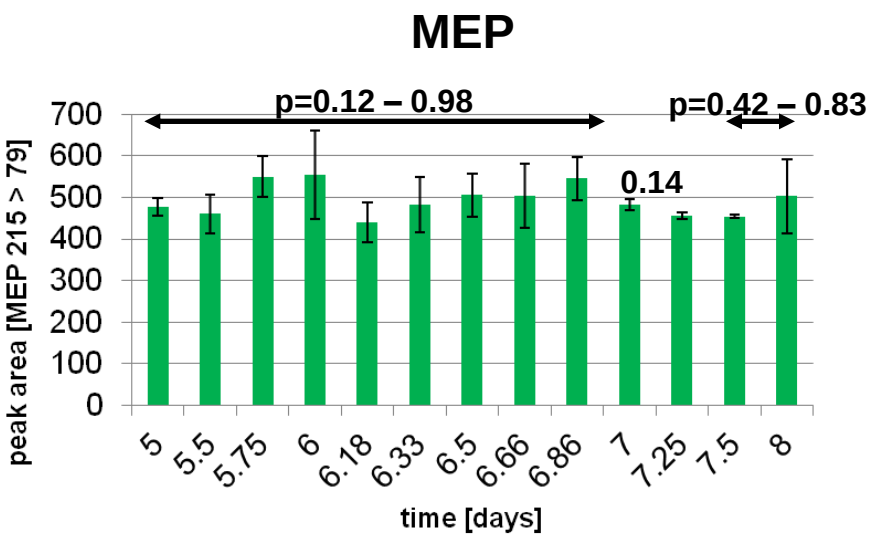
12C-glucose
13C-glucose
Expon. (12C-glucose)
Expon. (13C-glucose)

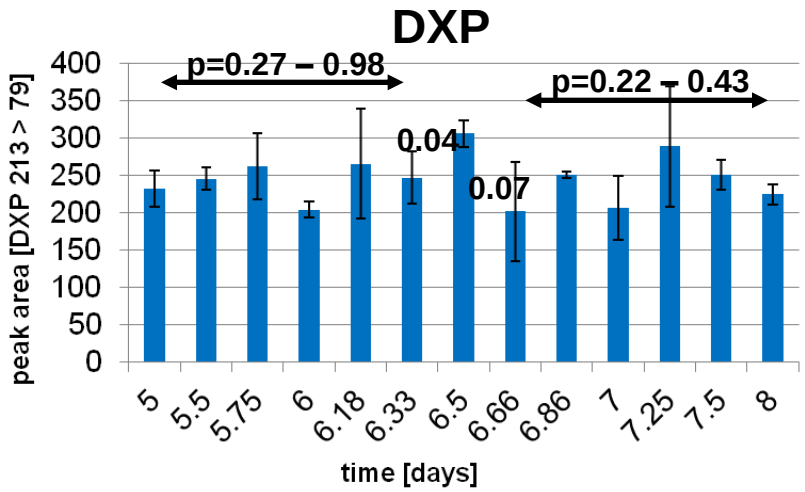
- **metabolic steady state**
- **feeding experiments**
- **sampling for isoprenoid analysis**

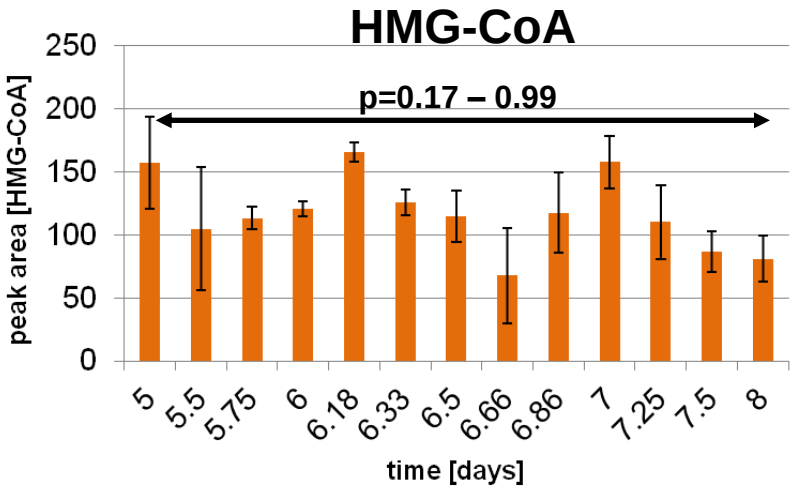
No.	day	date	time	No of samples
1	5	Tuesday	16:15	3
2	5.5	Wednesday	4:15	3
3	5.75	Wednesday	10:15	3
4	6	Wednesday	16:15	3
5	6.166	Wednesday	20:15	3
6	6.333	Thursday	0:15	3
7	6.5	Thursday	4:15	3
8	6.666	Thursday	8:15	3
9	6.833	Thursday	12:15	3
10	7	Thursday	16:15	3
11	7.25	Thursday	22:15	3
12	7.5	Friday	4:15	3
13	8	Friday	16:15	3
				39

Metabolic steady state – MEP+MVA pathway

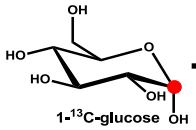








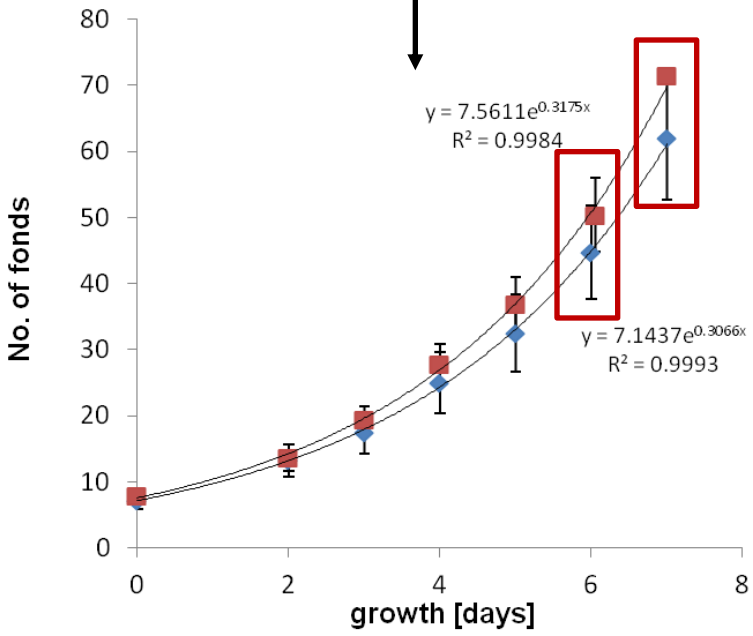
Isotopic steady state – MEP+MVA pathway

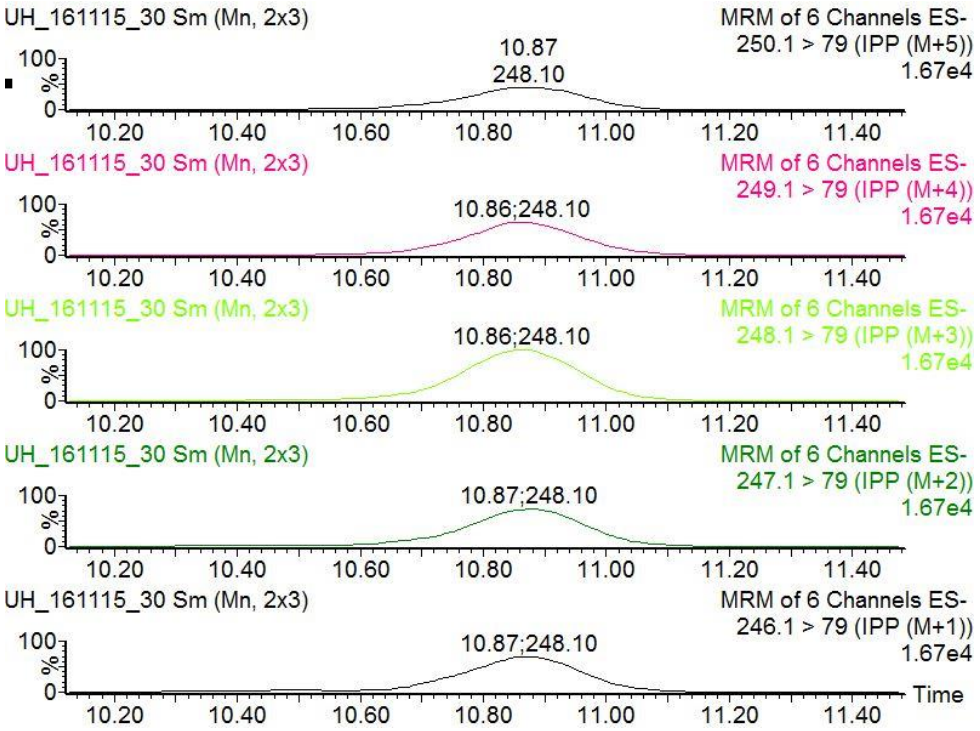


U-^{13}C-glucose

Spirodela polyrrhiza

$^{12}CO_2$

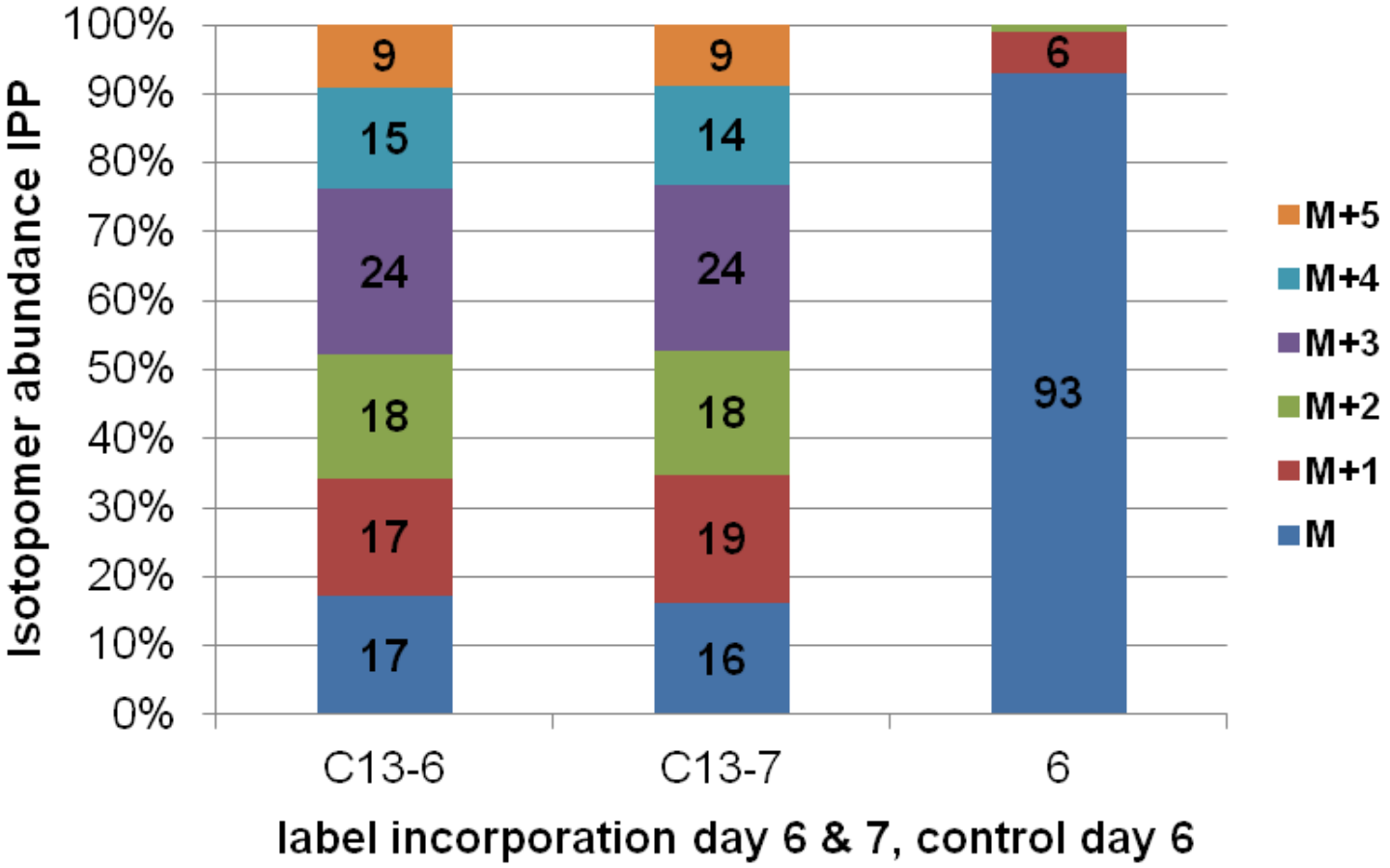




➢**MRMs for all possible isotopomers**

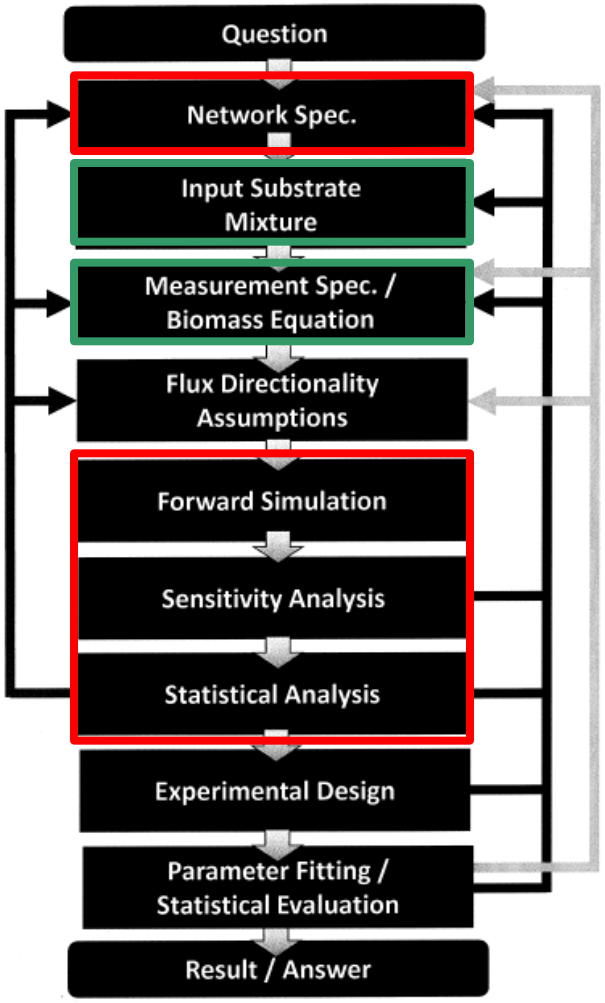
WEIZMANN INSTITUTE OF SCIENCE

Isotopic steady state – MEP+MVA pathway



WEIZMANN INSTITUTE OF SCIENCE

Flux analysis – ongoing experiments



- *labeling experiments with U-13C-glucose*
- *labeling experiments with 1-13C-glucose*
- *end product measurements*
- *treatments with inhibitors (Me jasmonate, Statins)*

- *forward simulations*
- *measurement of precursors / constrain influx*
- *flux calculations*

Summary

- ***Weizmass & Matchweiz** for identification of natural products*
- ***Labelling techniques** can assist identification*
- ***spacial resolution** as another dimension*
- ***Lemna as a model system for ^{13}C-MFA in “whole plants”***

Acknowledgments

Nir Shahaf

Jedrzej Szymanski

Yonghui Dong

Liron Feldberg

Sagit Meir

Ilana Rogachev

Sergey Malitsky

Asaph Aharoni

& the lab

& you for listening