IECM
3rd International Electronic Conference on Metabolomics
15-30 November 2018
chaired by Prof. Peter Meikle, Dr. Thusitha W. Rupasinghe, Prof. Susan Sumner, Dr. Katja Dettmer-Wilde
sponsored by metabolites

Integrated metabolome mining and annotation workflow accelerates specialised metabolite discovery

Justin J.J. van der Hooft et al.

Bioinformatics Group – Wageningen University, The Netherlands

3rd International Electronic Conference on Metabolomics 15-30 Nov. 2018

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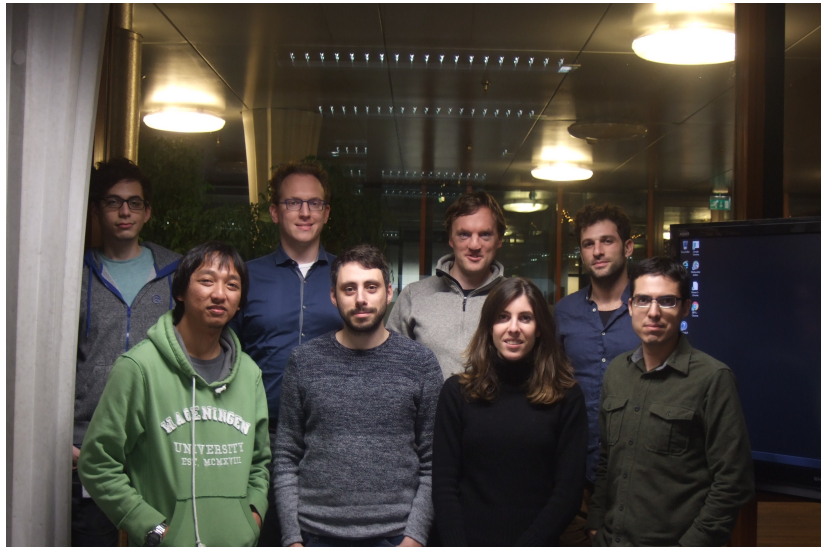
100years



Team work! ☺

Medema lab - Wageningen University, NL

Dorrestein lab – San Diego, USA

Glasgow Polyomics – University of Glasgow, UK

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Funding €€ NWO eScience center €€

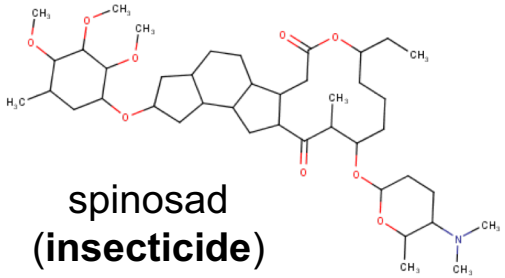
Glasgow Polyomics
www.glasgow.ac.uk/polyomics

University of Strathclyde Glasgow

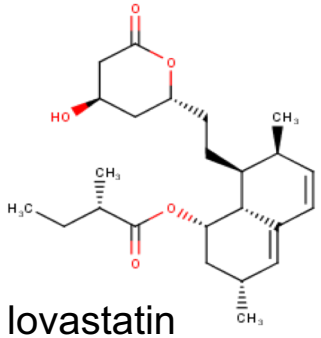
University of Glasgow

The challenge….

Bacteria, fungi, and plants produce a large & diverse arsenal of high-value molecules:

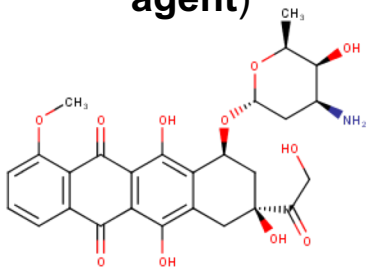


spinosad (**insecticide**)

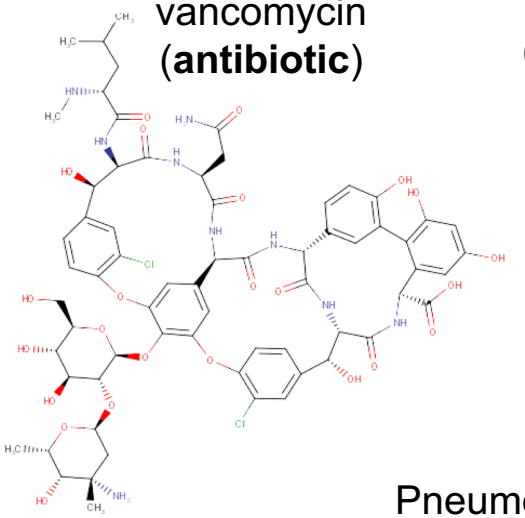


lovastatin (**cholesterol lowering agent**)

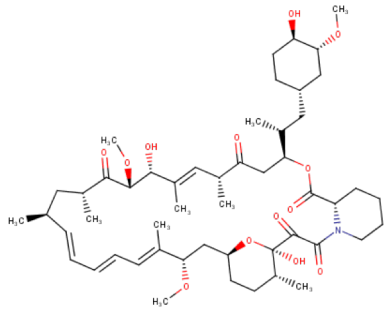
doxorubicin (**chemotherapeutic agent**)



vancomycin (**antibiotic**)

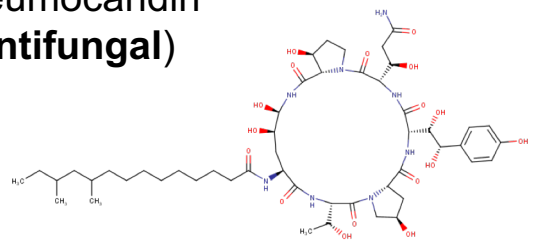


rapamycin (**immunosuppressant**)



Mass spectrometry fragmentation spectrum

Pneumocandin (**antifungal**)



….is **large-scale coupling of spectral data to molecular structures** of known & especially **novel** natural products molecules

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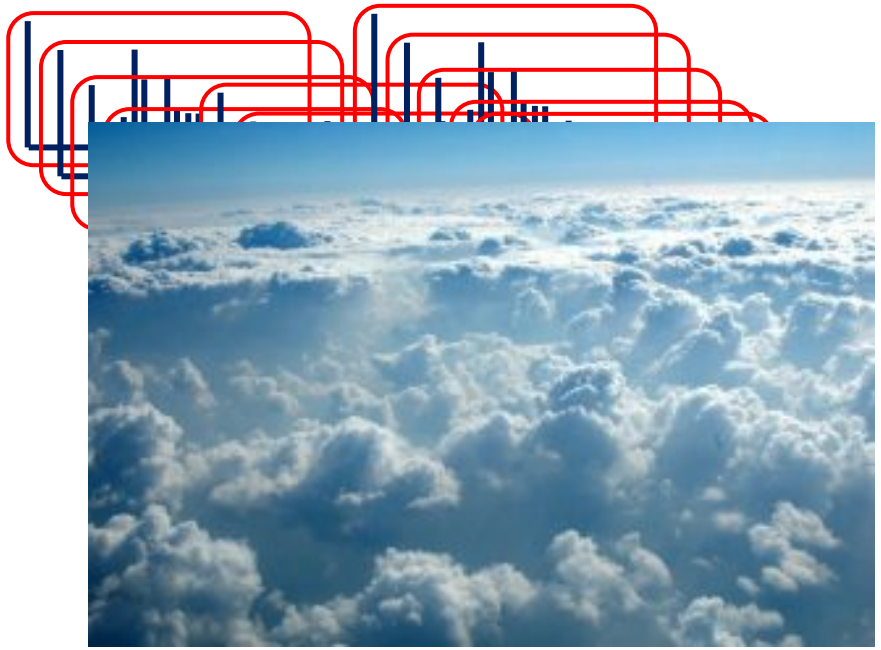
Motivation



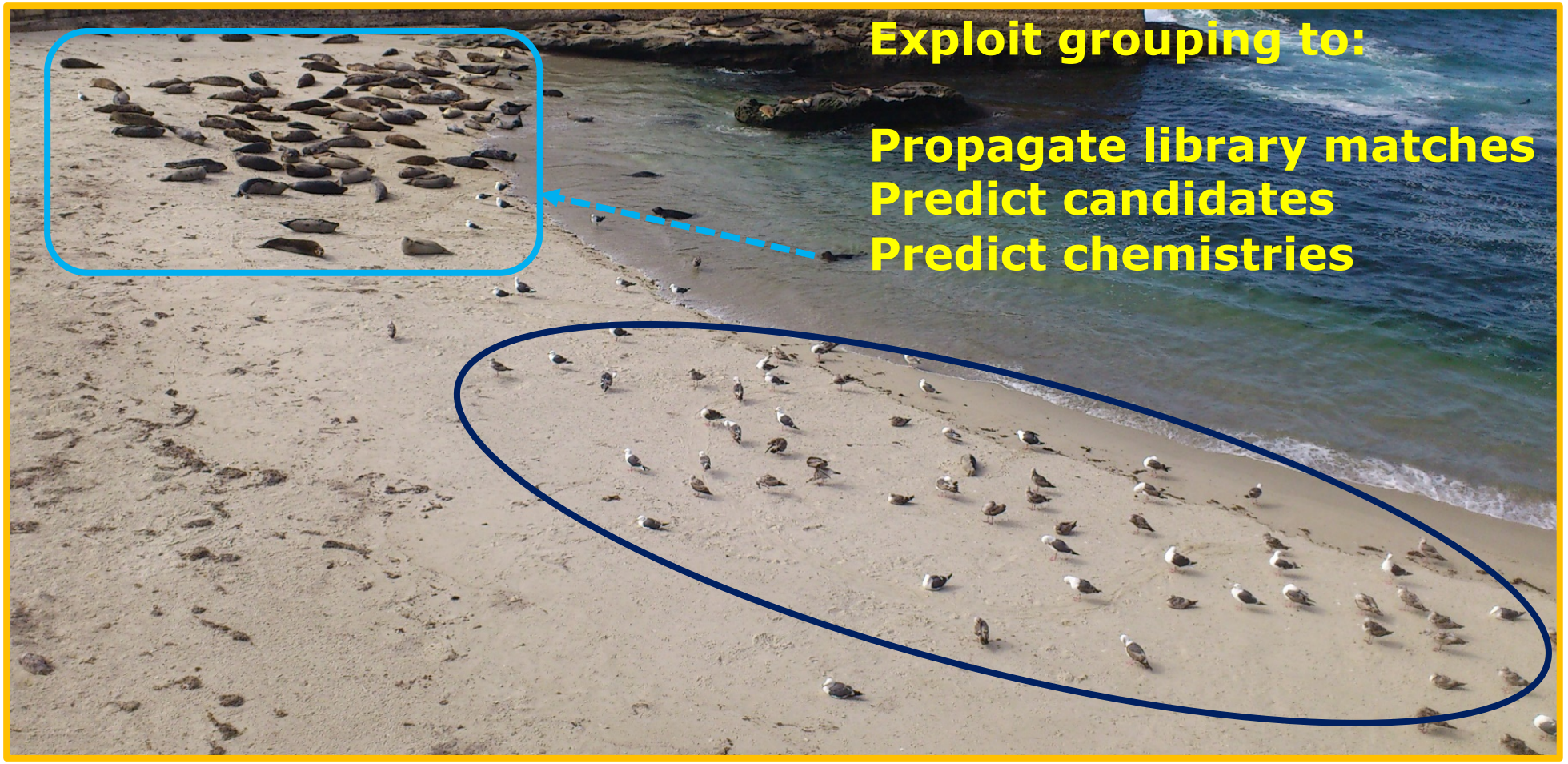
MS/MS information is key to study large sample sets with diverse chemistries

Integrated workflow exploits complementary tools to enhance interpretation

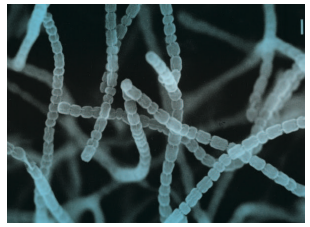
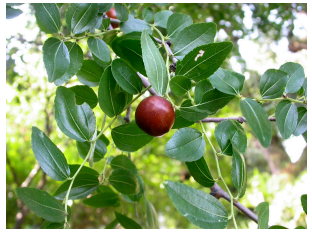
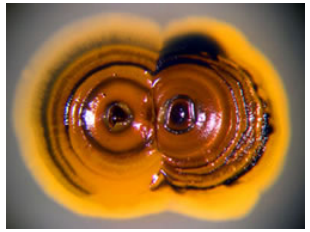
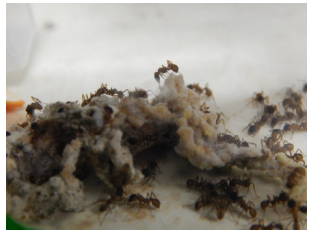
Motivation: Improved annotation power by pattern mining

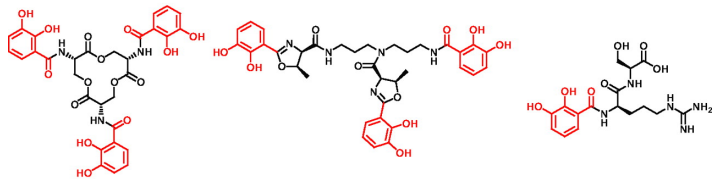
Molecular Families based on spectral similarity (Molecular Networking in GNPS)

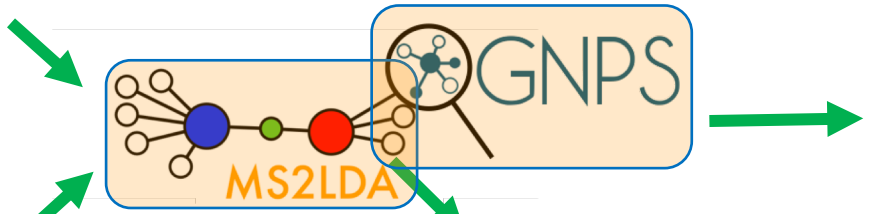
Substructures by extracting "building blocks of metabolomics" (MS2LDA)

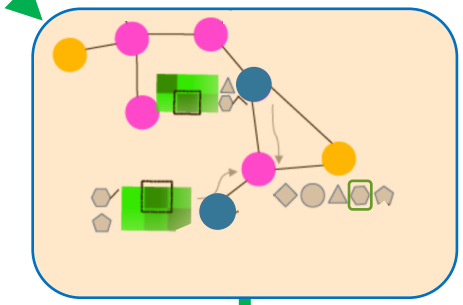


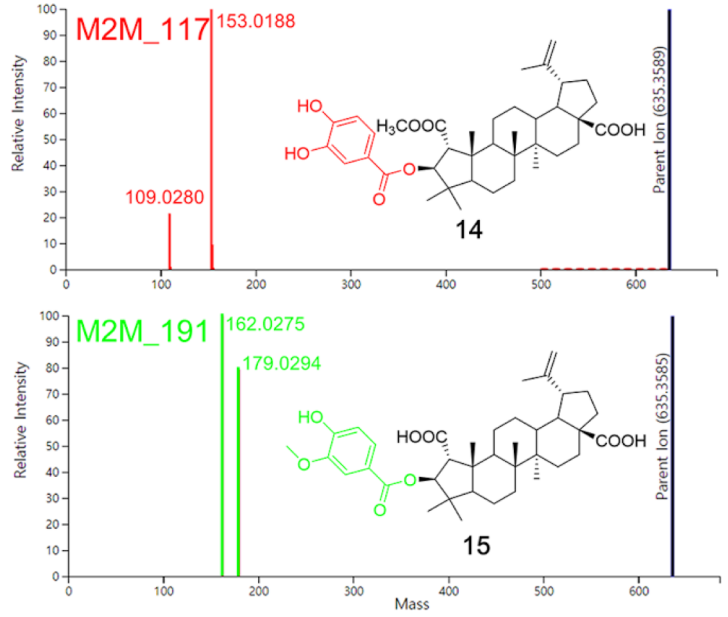
Integrated workflow

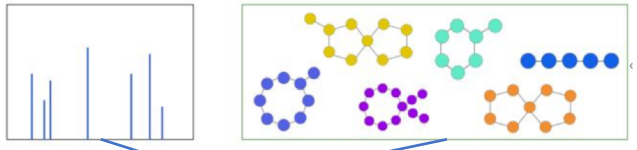





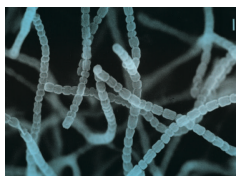


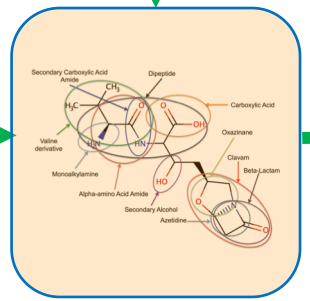


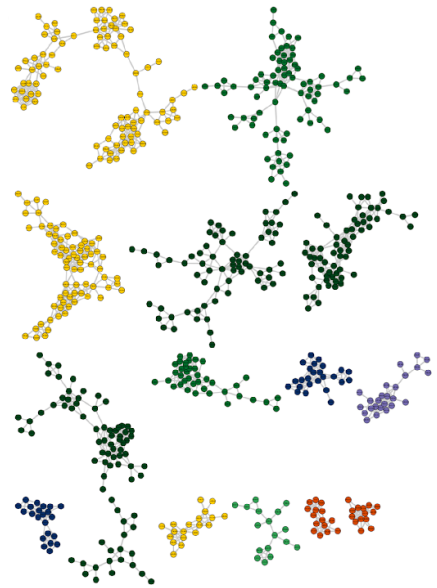












Dr Madeleine Ernst, UCSD

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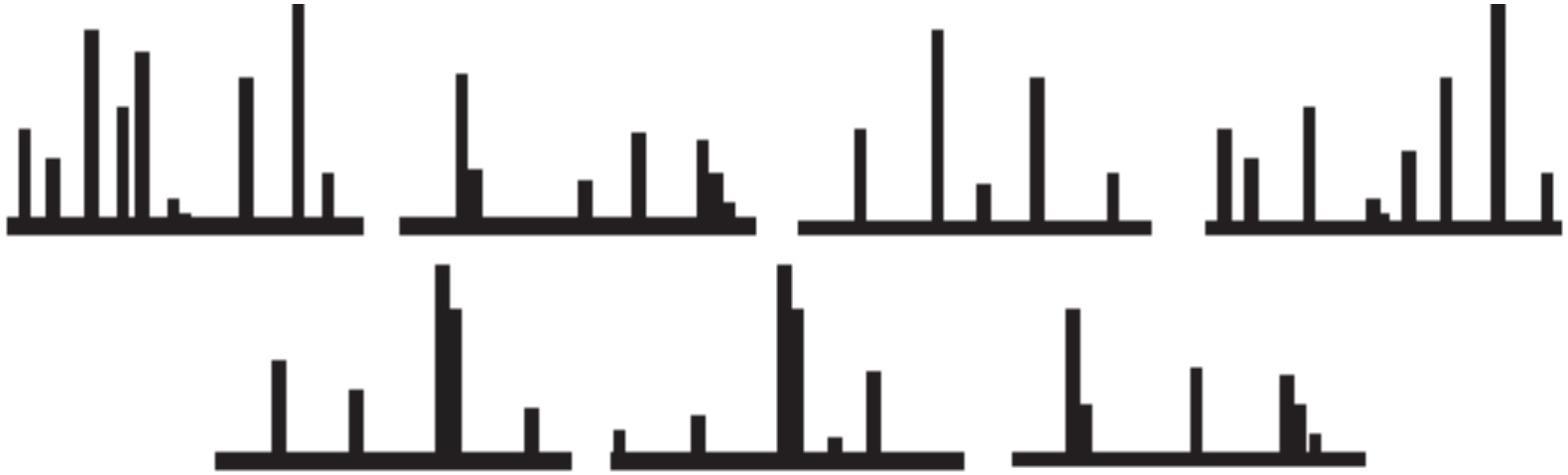


Molecular Networking

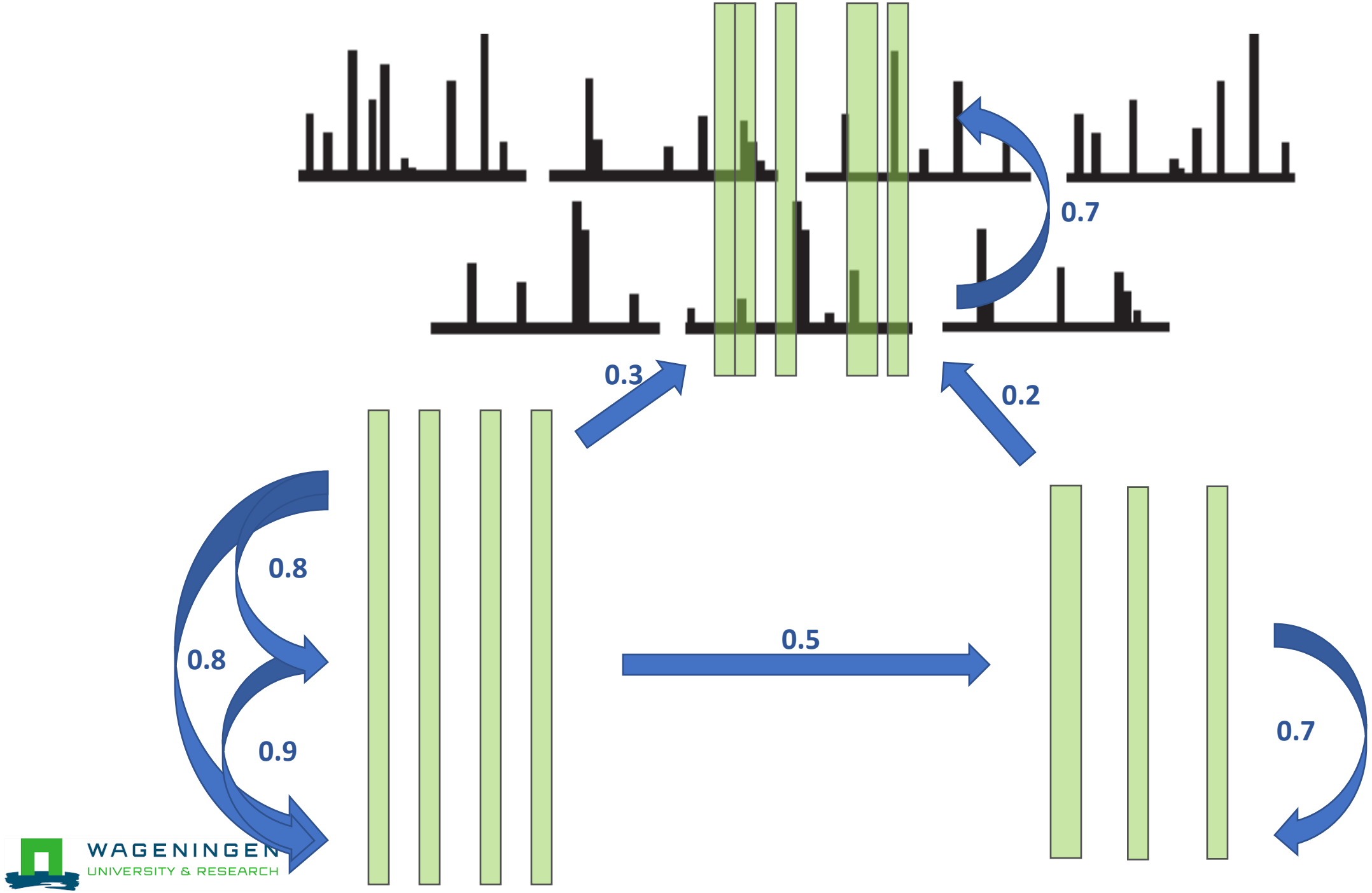
Very similar MS/MS spectra are grouped to:

- link spectra across different samples
- reduce redundancy in data set (”consensus spectrum”)

Wang, M., et al., *“Sharing and community curation of mass spectrometry data with Global Natural Products Social Molecular Networking”* Nat Biotech (2016)

Watrous, JD *et al*. “Mass spectral molecular networking of living microbial colonies” *PNAS* (2012)

Dr Ricardo R. da Silva
Dr Madeleine Ernst
Dr Mingxun Wang
Dr Louis-Felix Nothias



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Spectral library matches from GNPS

Libraries from diverse sources

Seed node annotations for molecular families

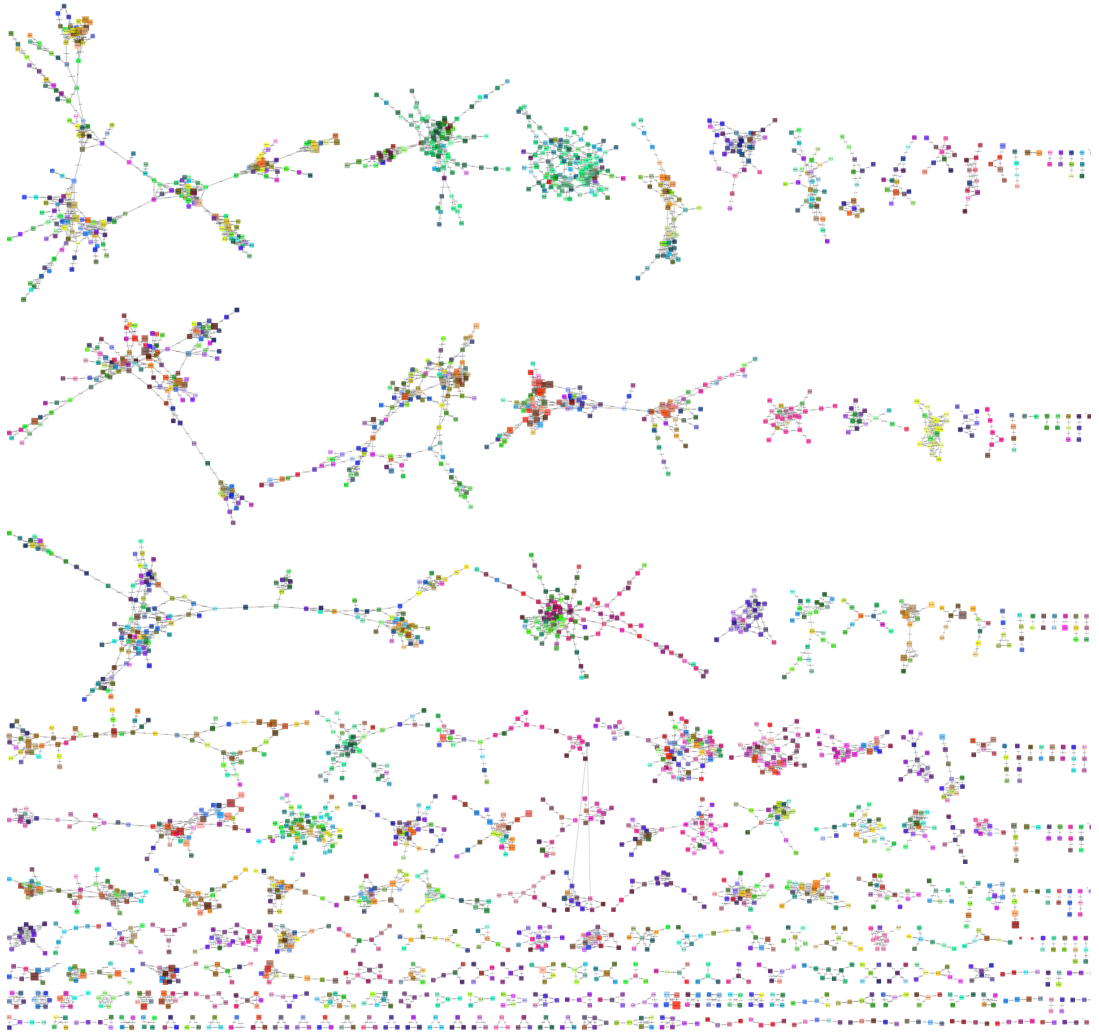
Library MS/MS Spectra

Diterpenoids

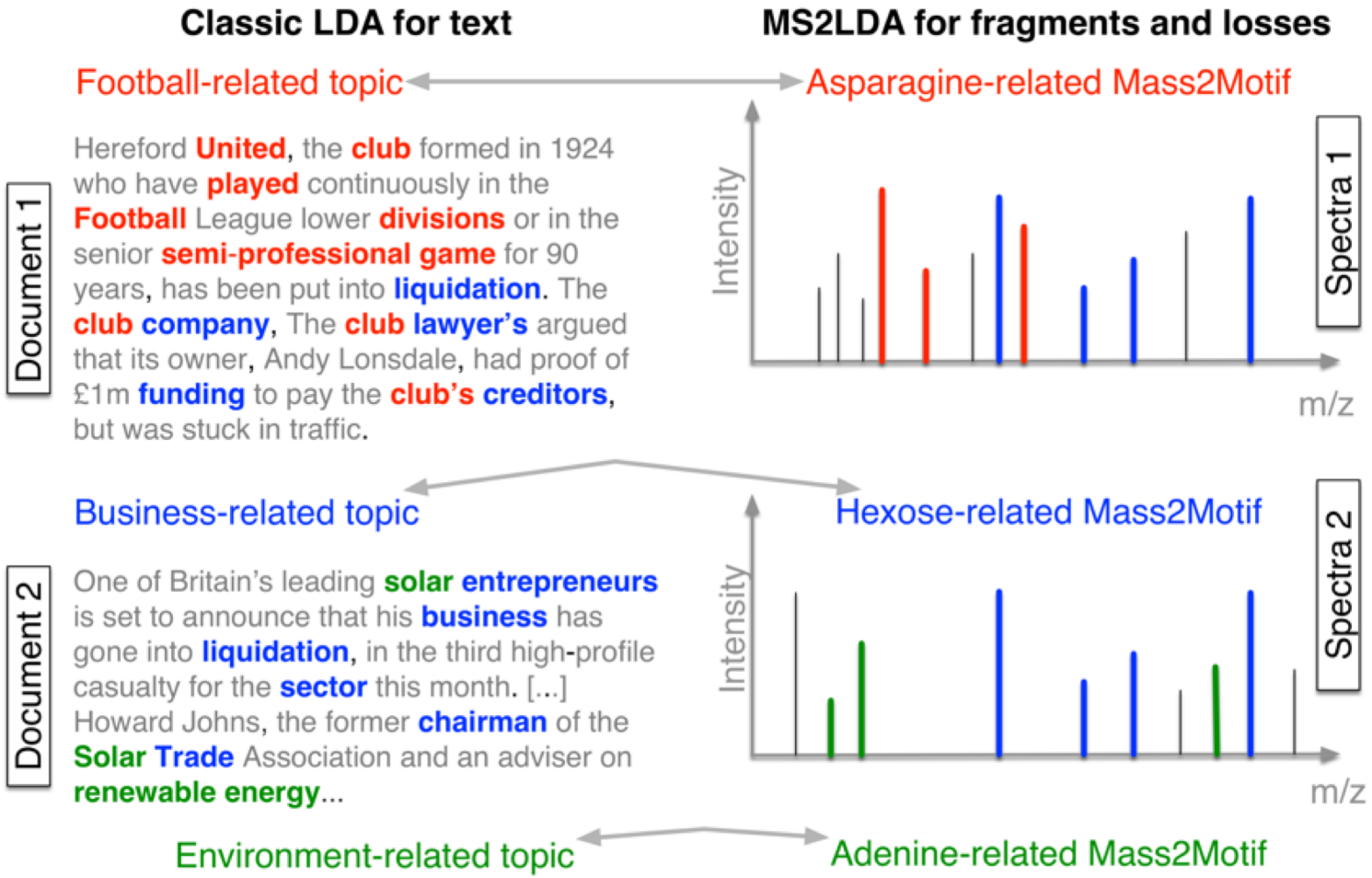
Flavonoids

Pharmaceuticals

MS contaminants

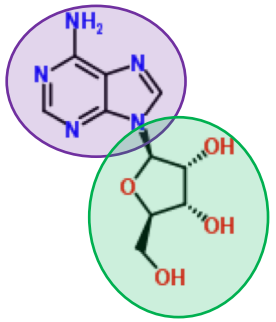


Topic modelling: from text to molecules



MS2LDA

Unsupervised Substructure Discovery



Documents <-> molecules

Words <-> fragments/neutral losses

Van der Hooft et al., PNAS, 2016

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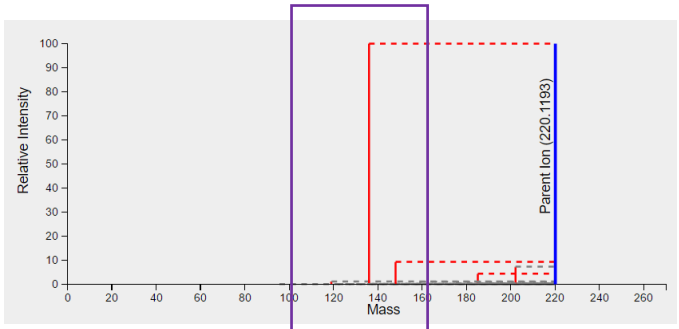
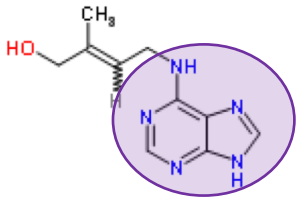
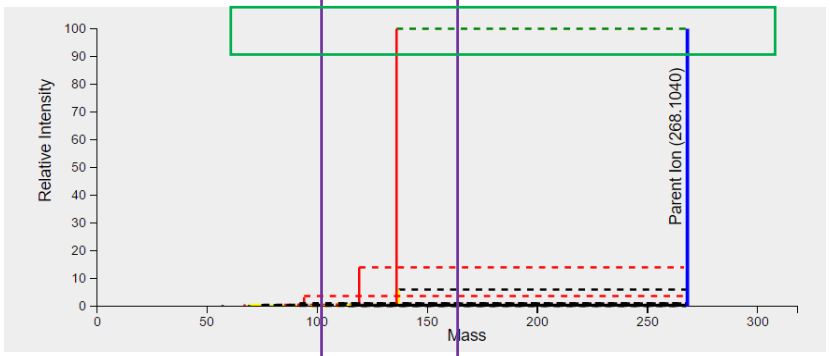
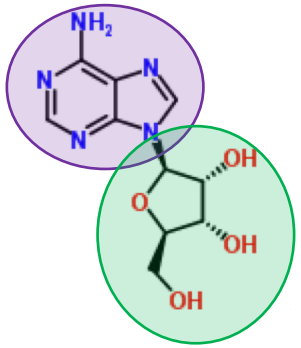
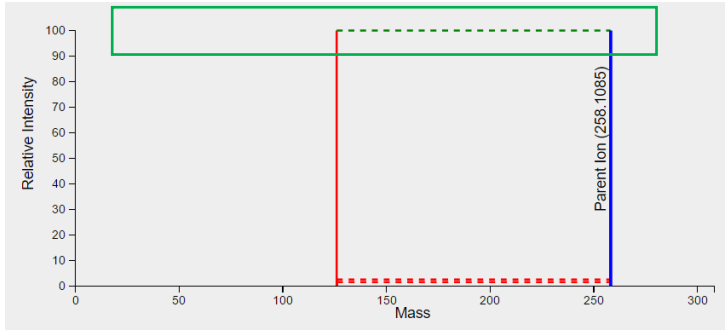
Validation: **MS2LDA** with standards

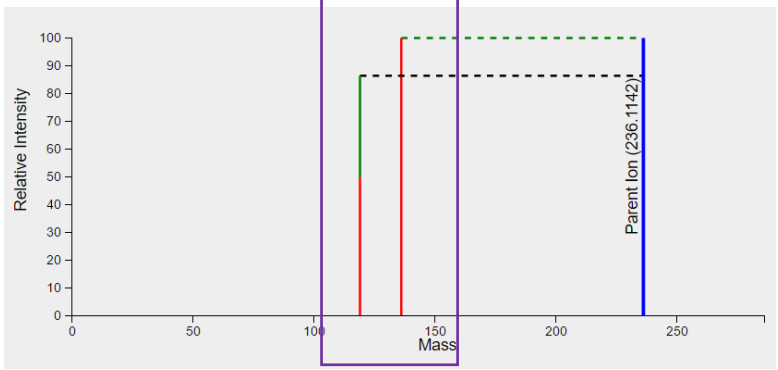


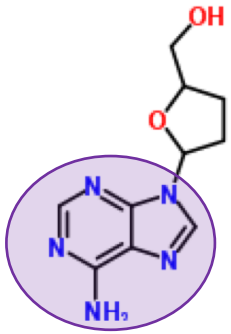


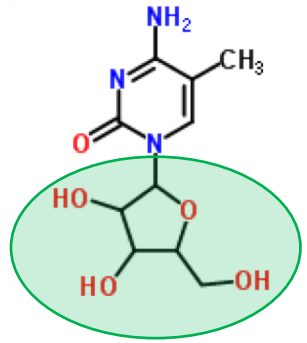


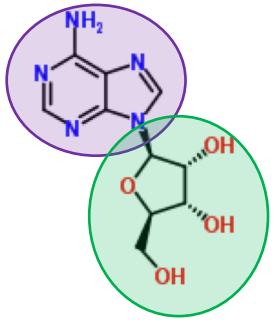












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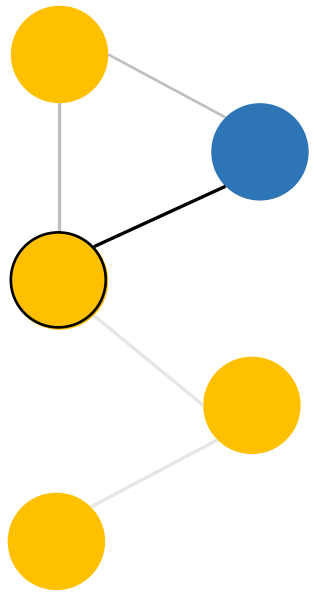
Network annotation propagation

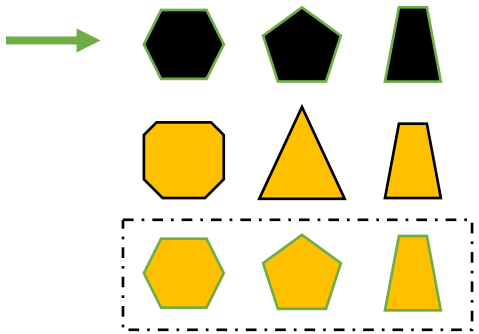
More coherent candidate structures within family

Rerank list based on **neighboring annotations**

Exploiting the **network topology**



Molecular Fingerprints

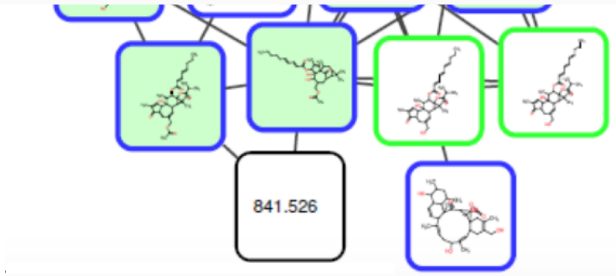


Ranked lists of in silico annotators (MetFrag)

Reranked(!) lists of in silico annotators

Library Match

Scans	Assigned	Candidate score	Molecule	Formula	Mass	ΔMass (ppm)	Name
1	No	2.23513		C13H16N2O5S	312.07799	0.86666	acetaminophen mercapturate (83967)
1	No	2.23513		C13H16N2O5S	312.07799	0.86666	(56923619)
1	No	3.20109		C13H16N2O5S	312.07799	0.86666	AKOS019768418 (81197200)
1	No	3.22166		C13H16N2O5S	312.07799	0.86666	CHEMBL1642089 (53321314)
1	No	3.22166		C13H16N2O5S	312.07799	0.86666	CHEMBL1642098 (53318691)
1	No	3.34424		C13H16N2O5S	312.07799	0.86666	AGN-PC-0G4R64 (64889340)
1	No	3.42103		C13H16N2O5S	312.07799	0.86666	AGN-PC-0DZQ65 (61409875)
1	No	3.42875		C13H16N2O5S	312.07799	0.86666	AGN-PC-0E3QTW (61600272)



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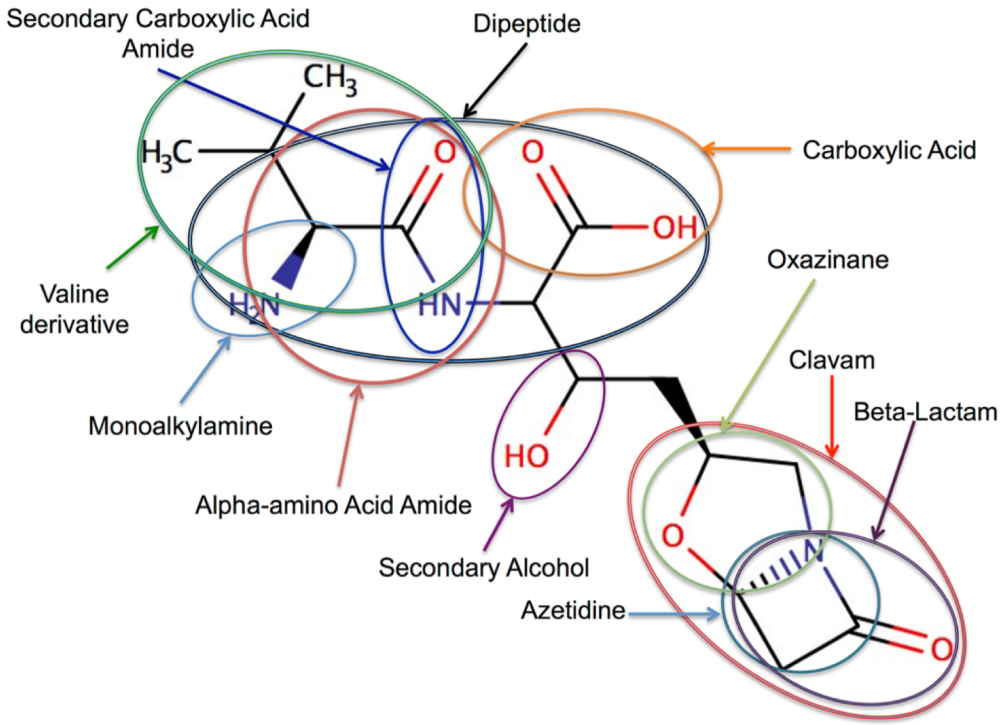
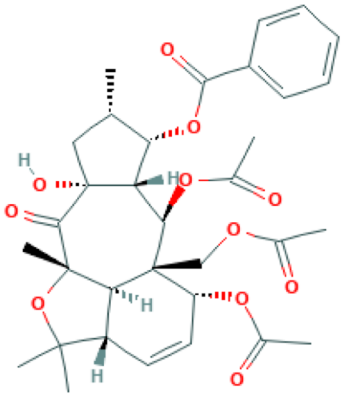
Da Silva et al., Plos Comp Biol., 2018

ClassyFire – SMILES and substituents





INPUT:

CC1CC2(C(C1OC(=O)C3=CC=CC=C3)C(C4(C(C=CC5C4C(C2=O)(OC5(C)C)C)OC(=O)C)COC(=O)C)OC(=O)C)O

OUTPUT:

ClassyFire

Kingdom: Organic compounds
Superclass: Lipids and lipid-like molecules
Class: Prenol lipids
Subclass: Diterpenoids
...

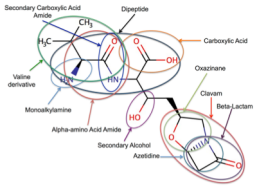
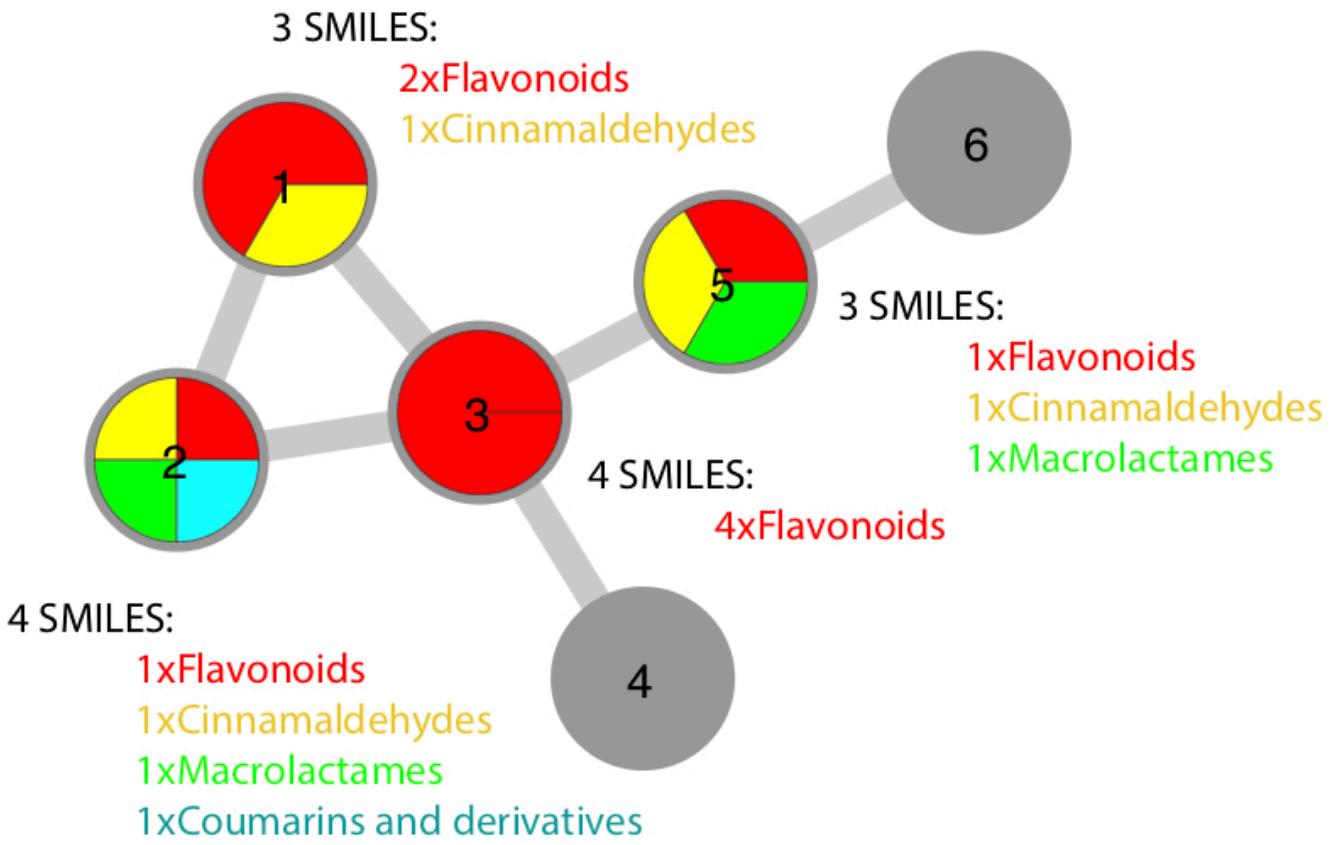


Figure part of Fig. 1 from Djoumbou Feunang et al., J. Cheminform, 2016

Djoumbou Feunang et al., J. Cheminform, 2016

ClassyFire – Chemical predictions for MFs



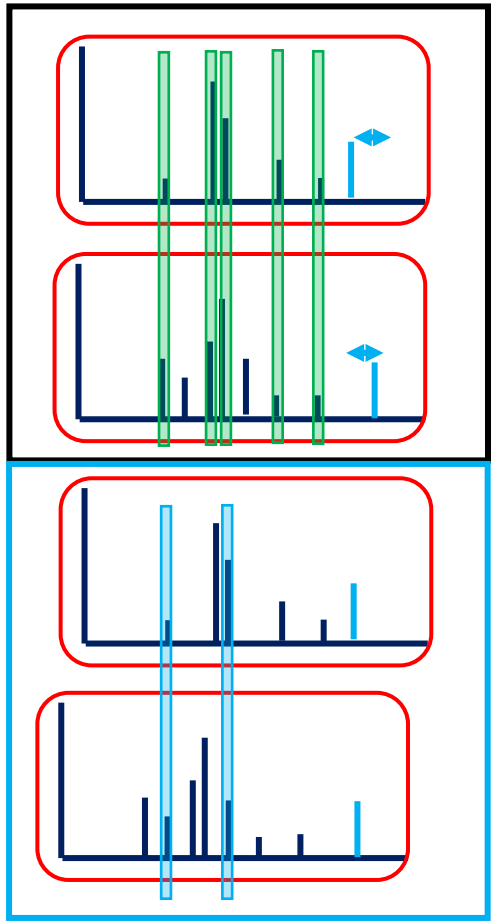
ClassyFire Scoring:

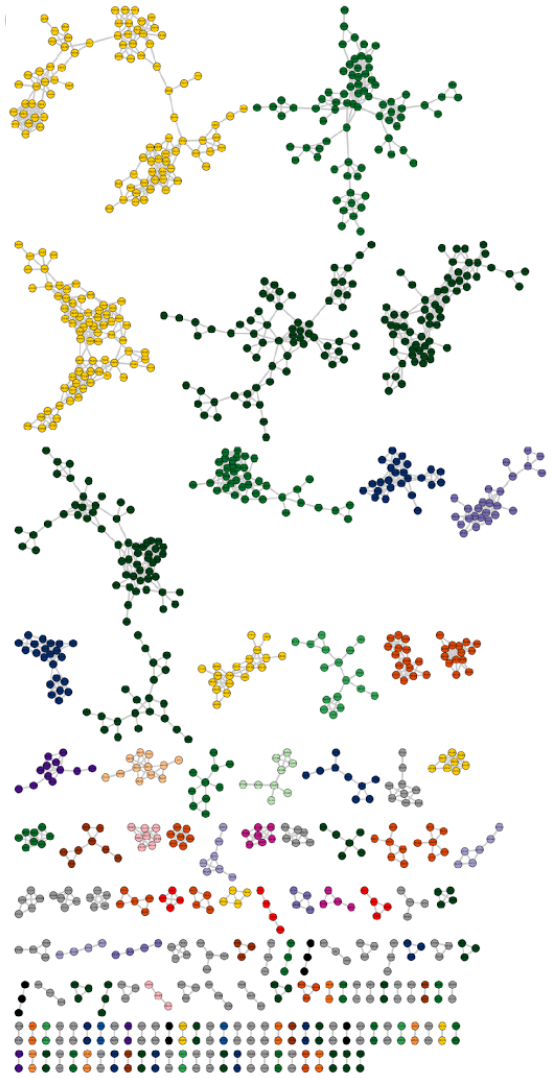
Flavonoids:	Database hits:
2.25 nodes/	4 nodes/
6 nodes =	6 nodes =
0.375	**0.67**

Disclaimer: scores dependent on structural database contents!

Illuminating the Rhamnaceae chemistry

Molecular Networking





- triterpenoids
- triterpenoidal saponins
- steroidal saponins
- flavonoid 3-*O*-glycosides
- flavonoid 7-*O*-glycosides
- flavonoid *O*-glycosides
- flavones, flavonones, flavonols
- 8-methylated flavonoids
- (hydroxyl)anthraquinones
- xanthones
- hydrolyzable tannins
- phenolic glycosides
- coumaric acid and derivatives
- lignan glycosides
- iridoid glycosides
- peptide
- hybrid peptide
- oligosaccharides
- long-chain fatty acids
- others / no matches

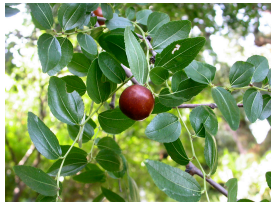
plant related classifications:

different flavonoids

phenolic glycosides

triterpenoids

Dr Kyo Bin Kang, UCSD



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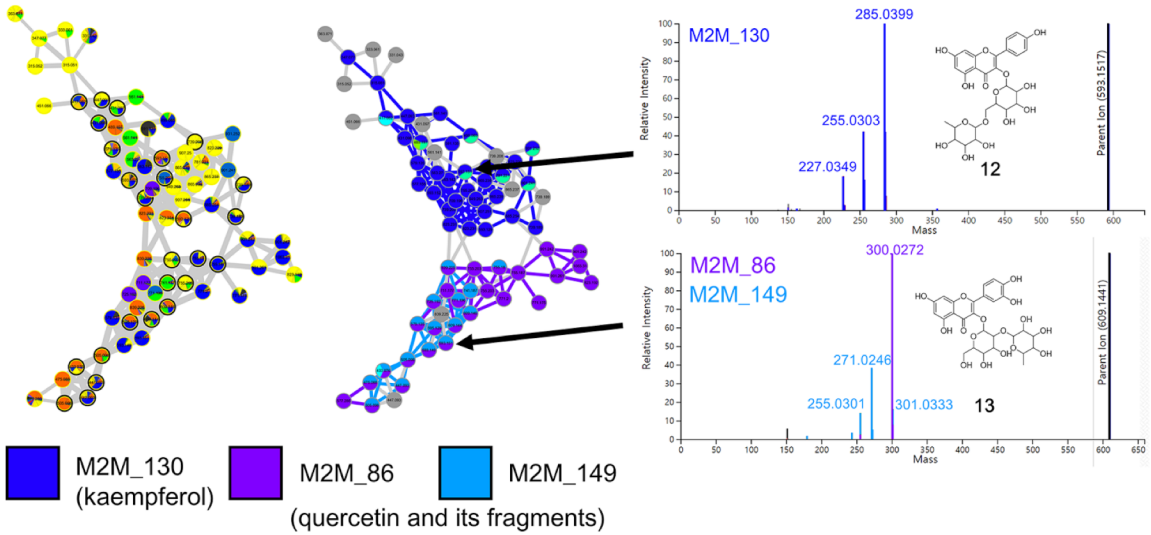
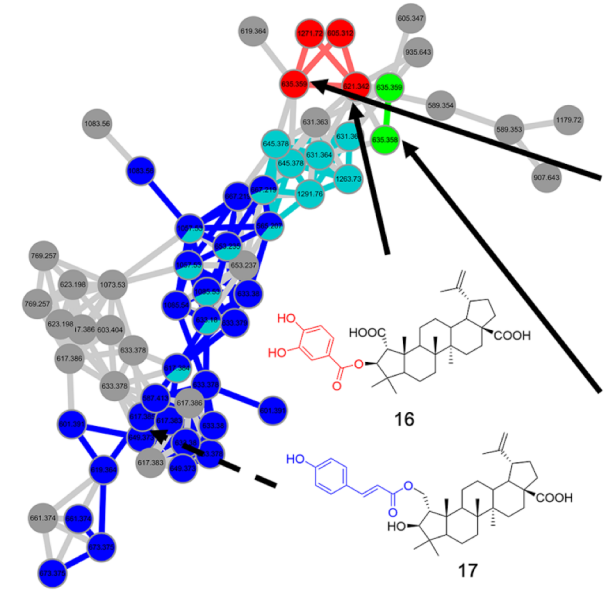
Annotating plant molecular families

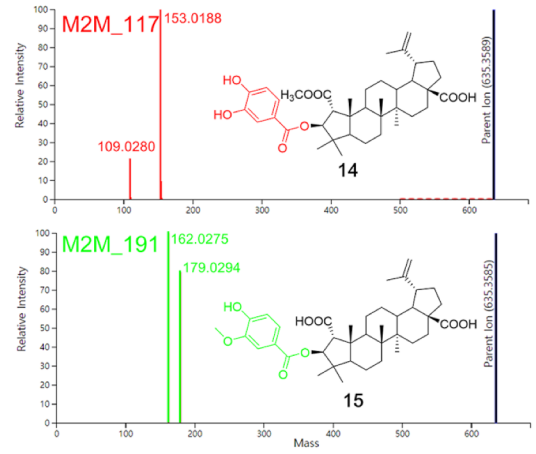


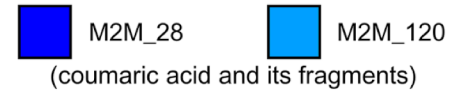
Flavonoid-3-*O*-glycoside Molecular Family:

Differentiation of subfamilies

Kaempferol and Quercetin based





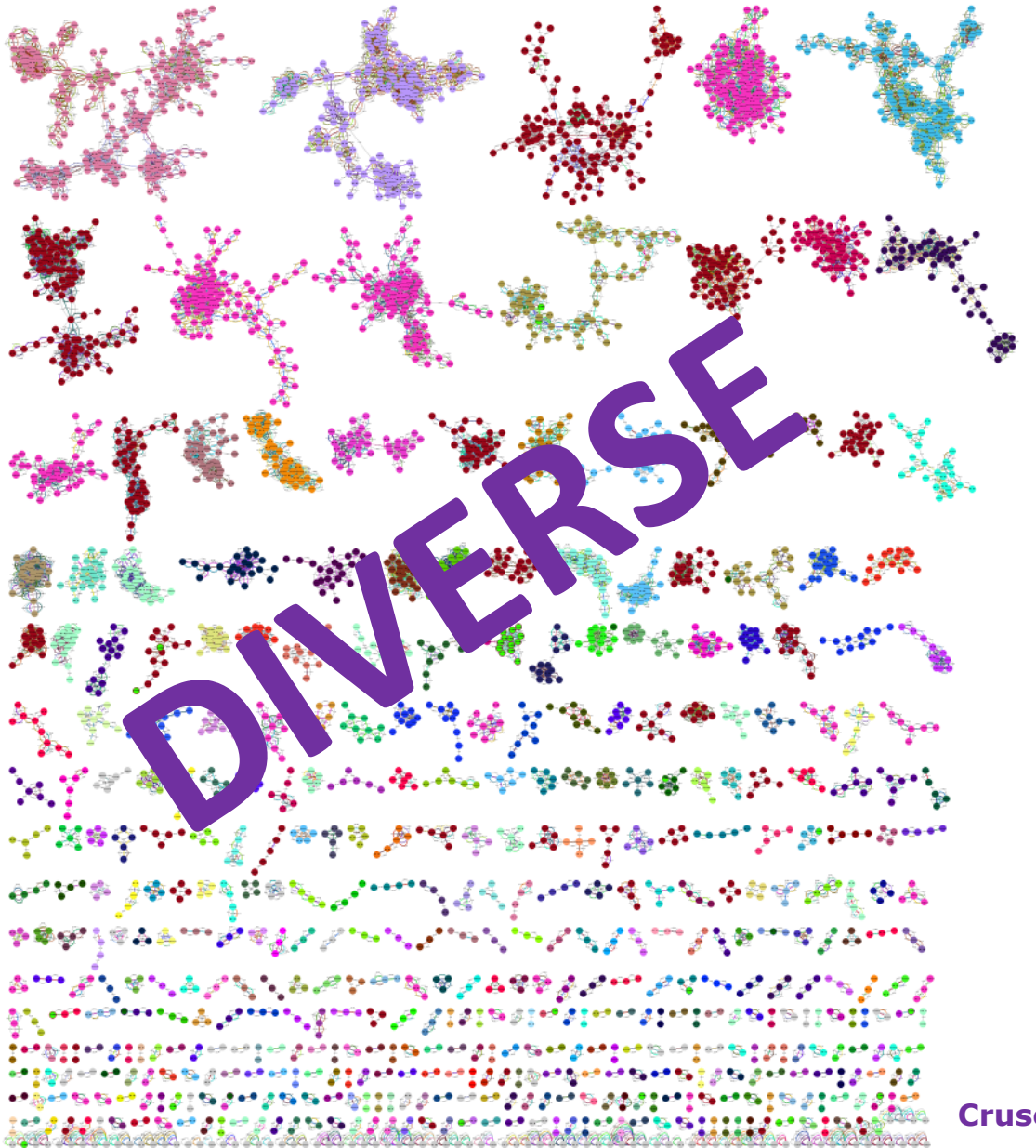


Triterpenoid Molecular Family:

Differentiation of modifications

Protocatechuic acid and Vanillic acid based

Illuminating bacterial chemistry



Molecular Network of
146 *Streptomyces* and *Salinispora sp*.

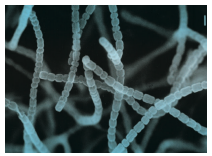
Triterpenoids

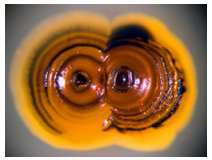
Lipids - PE

Cyclic peptides

Alpha amino acid esters

and many more….





Crusemann et al., J. Nat. Prod., 2017

Annotation of bacterial family I

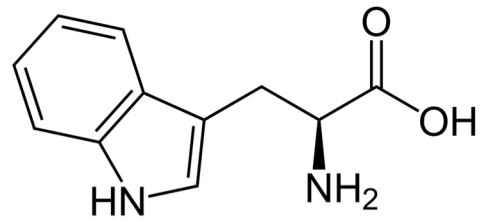
Mass2Motif Annotation

You can assign a label (annotate) this Mass2Motif from the **Annotation** field below. Additionally, a shorter annotation can also be assigned through the **Short Annotation** field. This will be used in the network visualisation.

Annotation: Tryptophan related Mass2Motif

Short Annotation: Tryptophan related Mass2Motif

Save

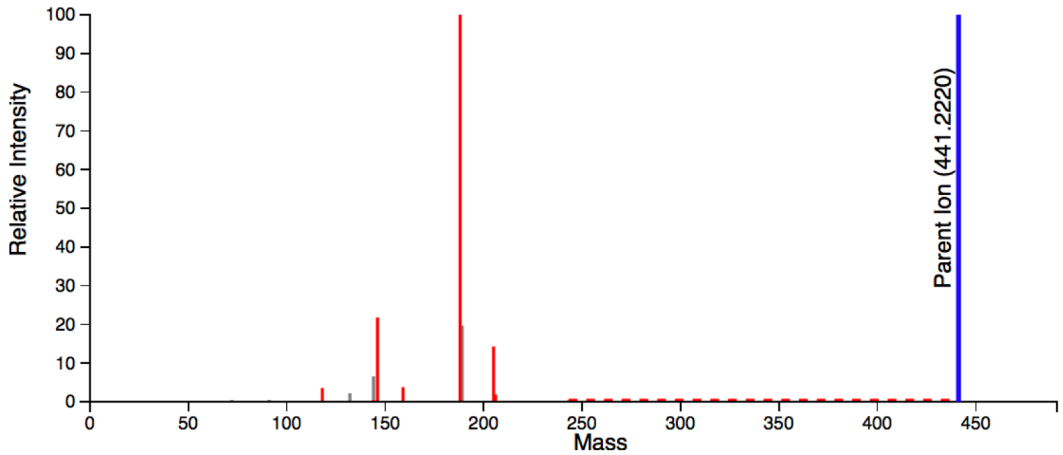


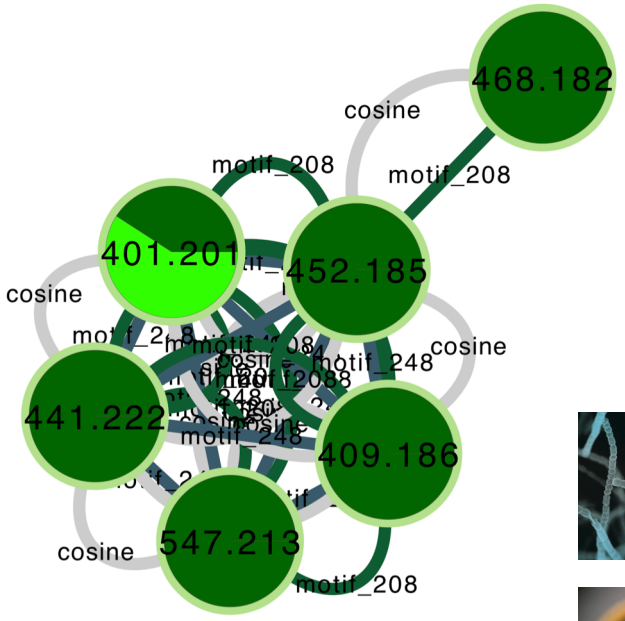
Motif 208

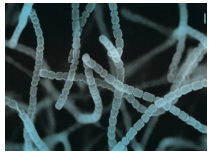
Previous

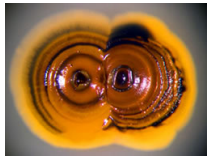
motif_208
Parent: document_1202, null (2/19)
Probability: Probability: 0.832888449166, overlap: 0.356384148972, Over

Next









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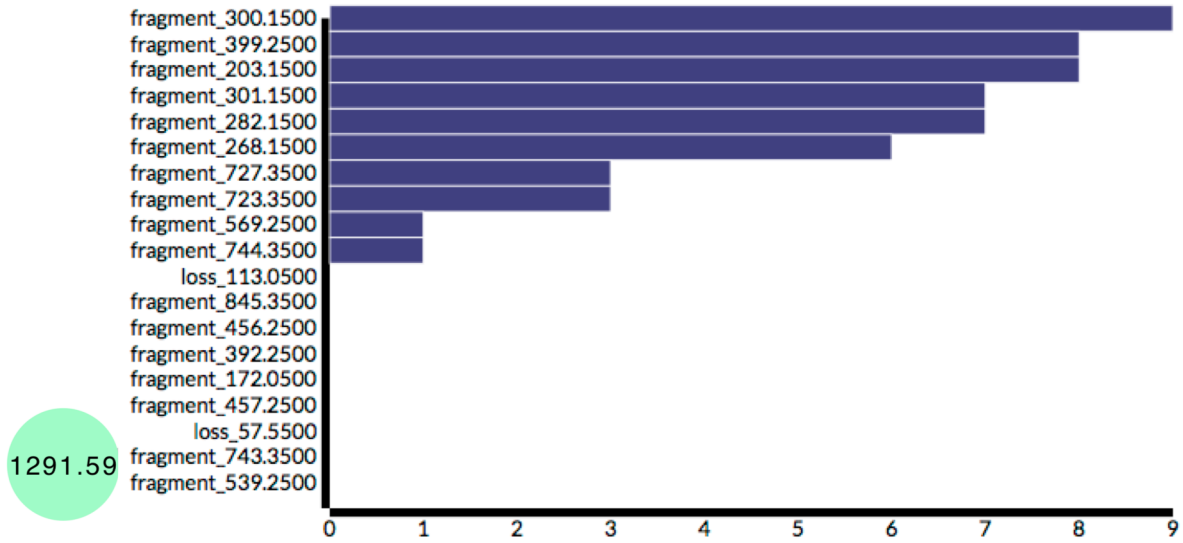
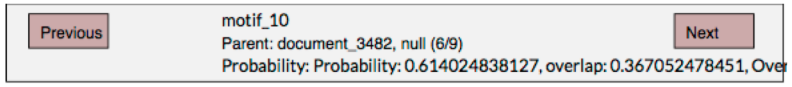
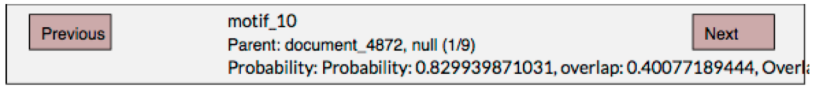
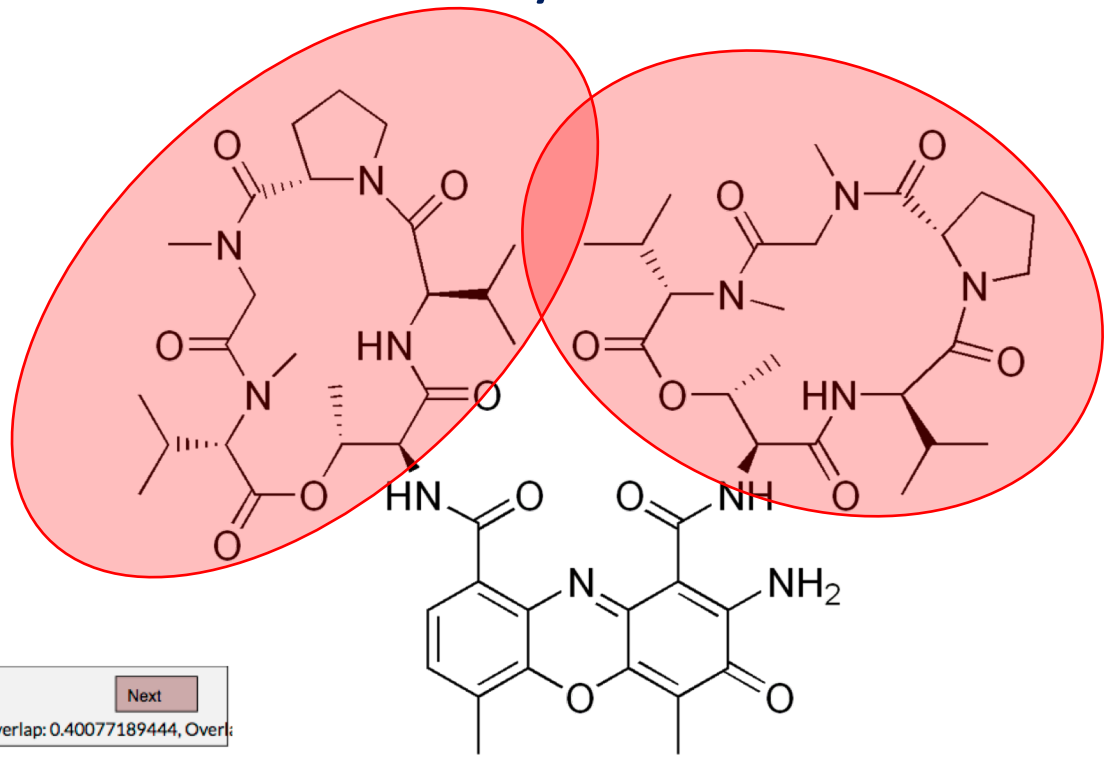
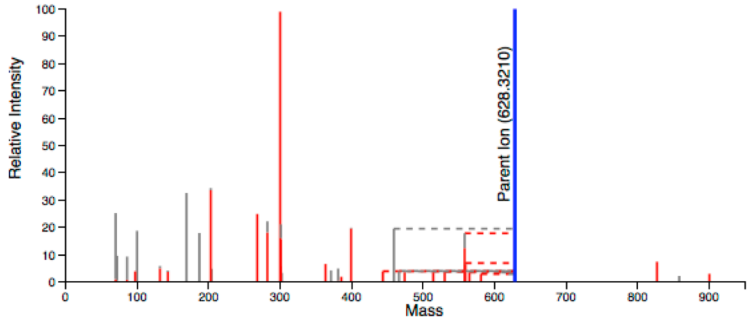
Annotation of bacterial molecular family II

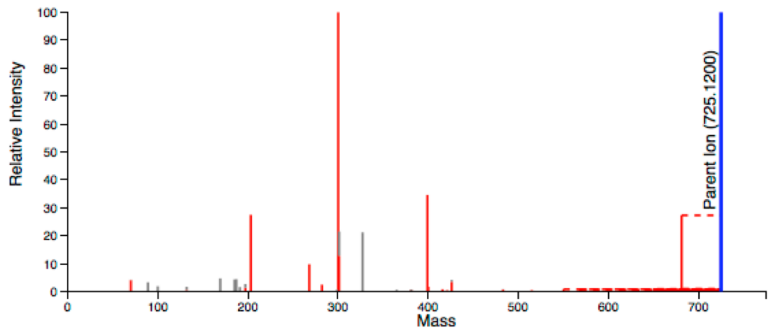






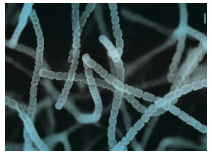


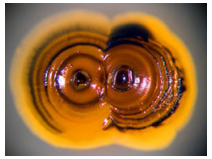




Actinomycin D

5	PeptideMass
.3E-7	1414.84
7E-10	1268.57
.0E-7	1290.57





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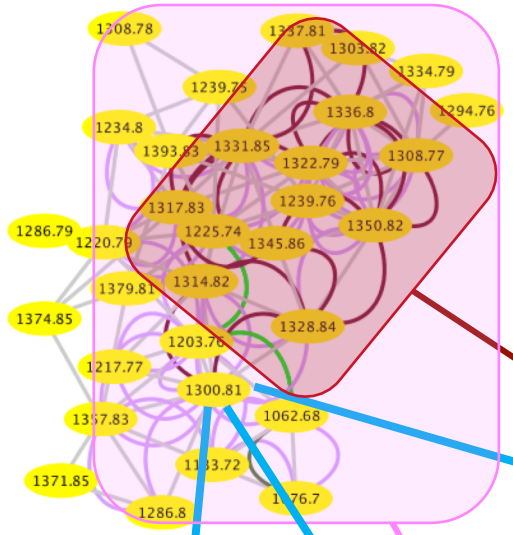
Mohimani et al., Nat. Chem. Biol., 2017

Crnovcic et al., RCS Advances, 2014



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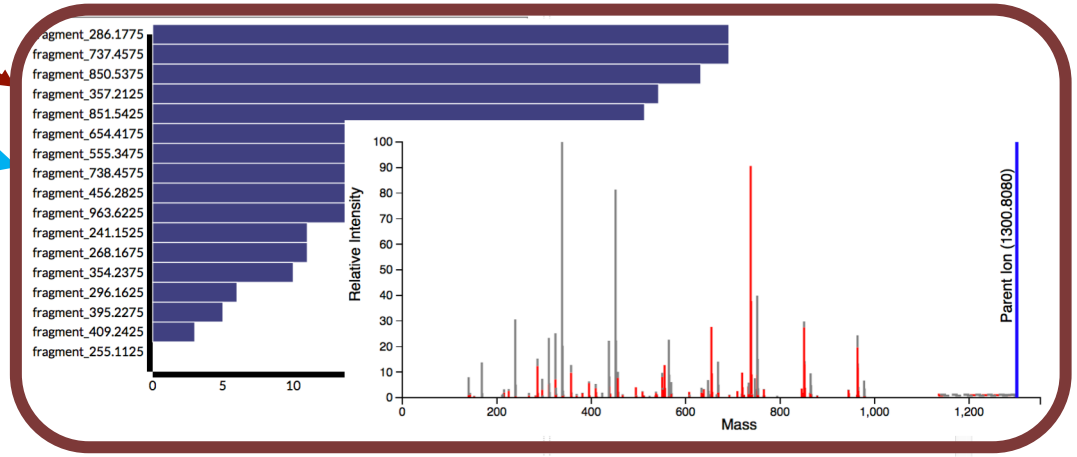
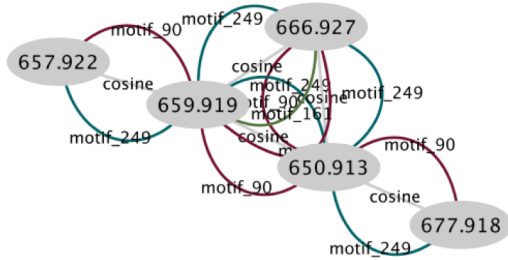
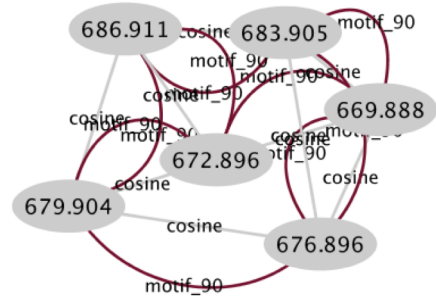
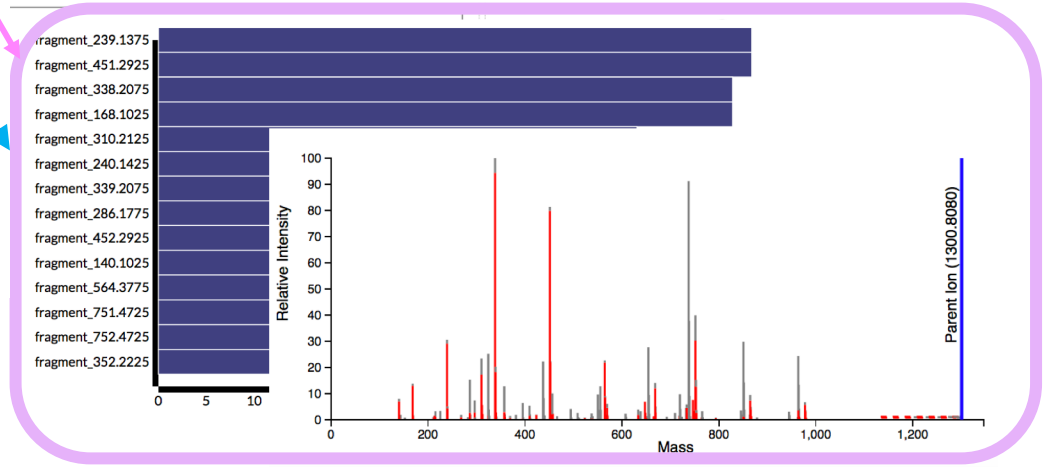
Xenoamicin_A_structure_0	Xenoamicin_A_structure_0	Xenoamicin_B_structure_1	Petriellin_A_Petriellin_A
		Xenoamicin_B_structure_1	Pentadactylin
		Xenoamicin_B_structure_1	Gramicidin_S2
		Xenoamicin_B_structure_1	Petriellin_A_Petriellin_A
Xenoamicin_B_structure_1	Xenoamicin_B_structure_1	Xenoamicin_B_structure_1	Xenoamicin_B_structure_1
Xenoamicin_A_structure_0	Xenoamicin_A_structure_0	Xenoamicin_A_structure_0	Xenoamicin_B_structure_1
	Cyclosporin,_9Cl_2-[O-(2-Hydroxyethyl...	Xenoamicin_B_structure_1	Xenoamicin_B_structure_1
		Xenoamicin_B_structure_1	Xenoamicin_B_structure_1
		Xenoamicin_B_structure_1	Xenoamicin_B_structure_1
Xenoamicin_B_structure_1	Xenoamicin_B_structure_1	Xenoamicin_B_structure_1	Xenoamicin_B_structure_1



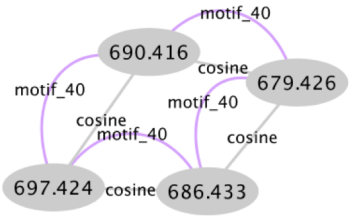


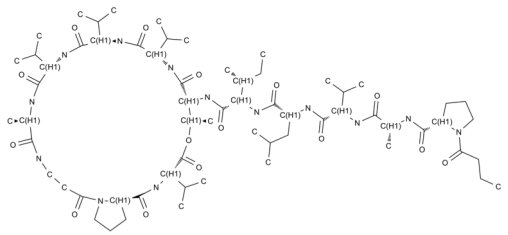
Ring



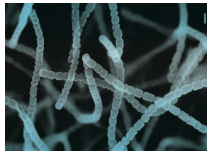


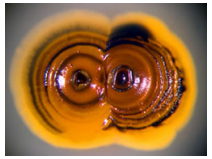
Tail





Xenoamicin A





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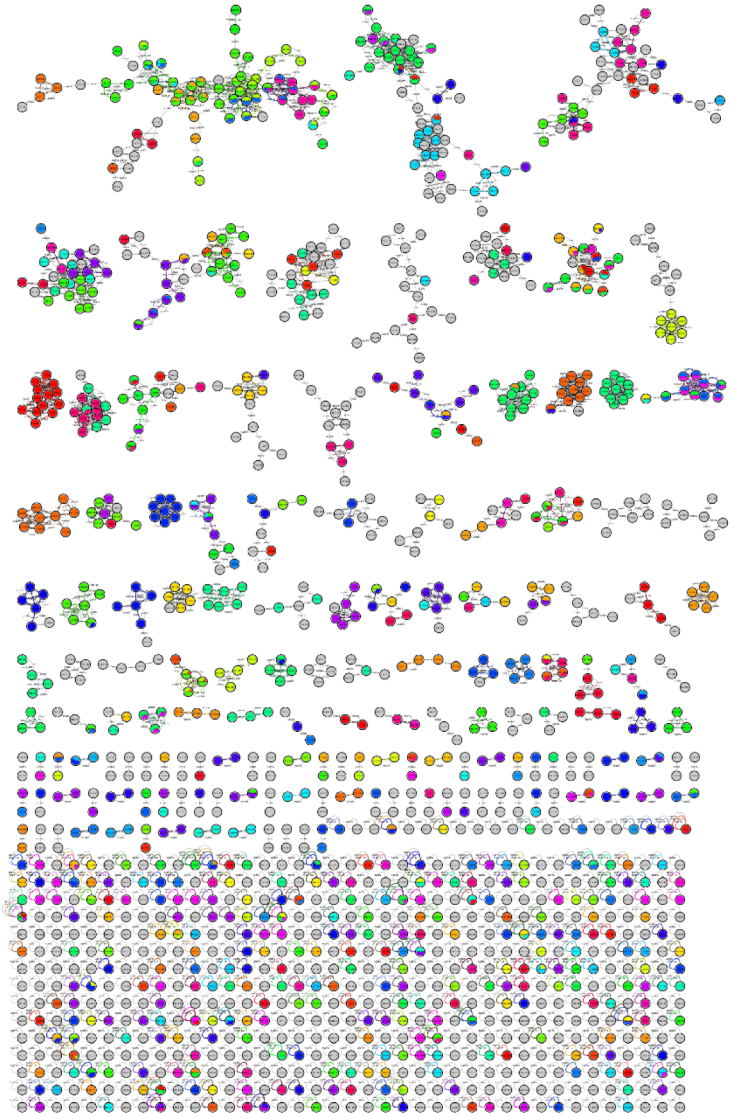
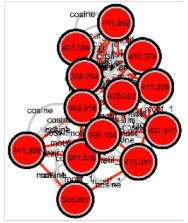
Annotation of fungal garden





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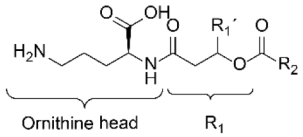
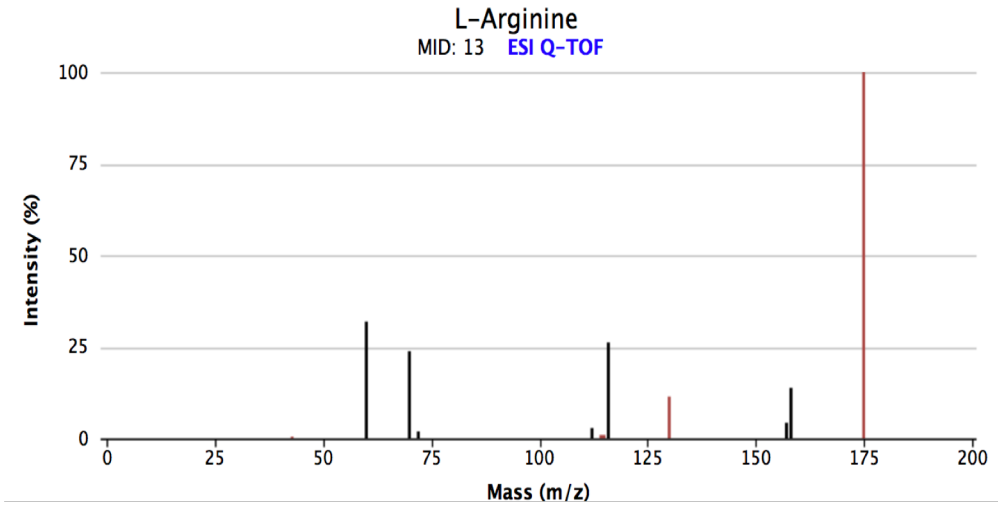
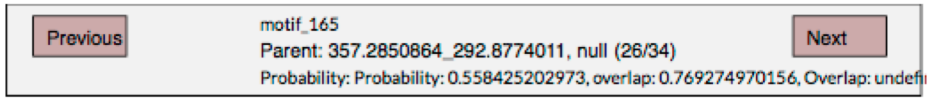
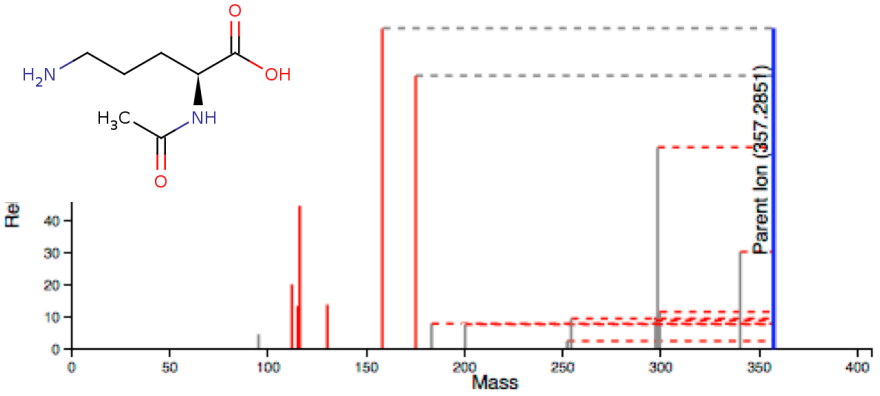




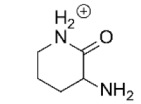


COLOR and SHAPE CODE

- **Motif 1 - N2-acetylornithine related**

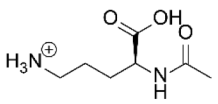


Fragment 115.0875

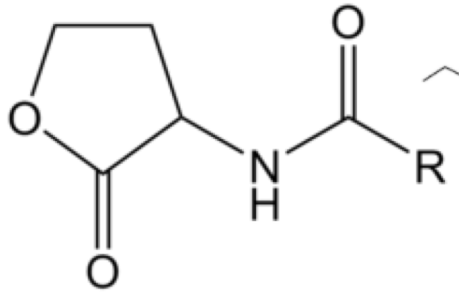


Exact Mass: 115.09

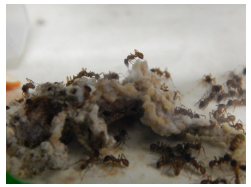
Fragment 175.1175



Exact Mass: 175.11



N-acyl homoserine lactone



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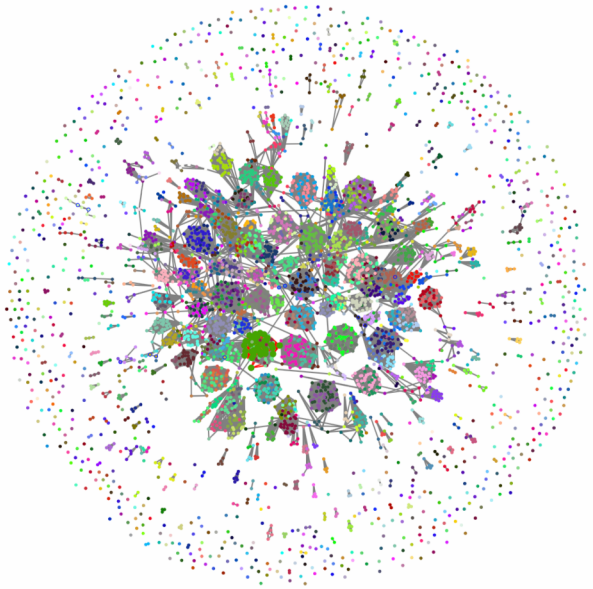
https://metlin.scripps.edu

Linking substructures to genetic elements

iOMEGA: Integrated Omics for MEtabolomics and Genomics Annotation

- Gene Cluster Families & Metabolite Families



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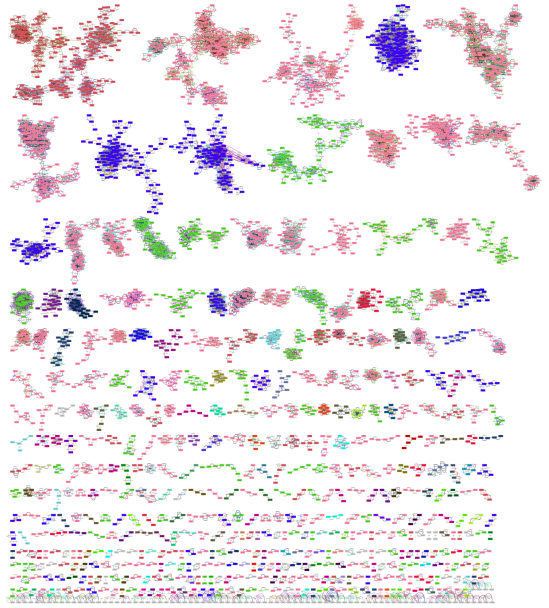

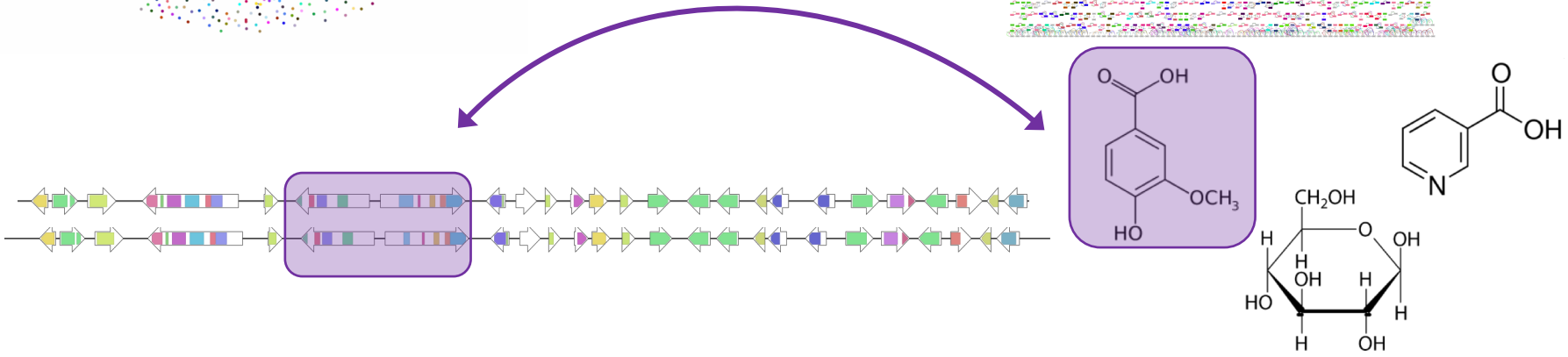

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Thank you!

Special Issue: Metabolomics Data Processing and Data Analysis—Current Best Practices

Deadline for manuscript submissions: **28 February 2019**

Guest Editors

Dr. Kati Hanhineva
University of Eastern Finland
Twitter: @KatiHanhineva

Dr. Justin van der Hooft
Wageningen University
Netherlands
Twitter: @jjjvanderhooft

Keywords
Metabolomics data processing data interpretation annotation and visualization data analysis

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