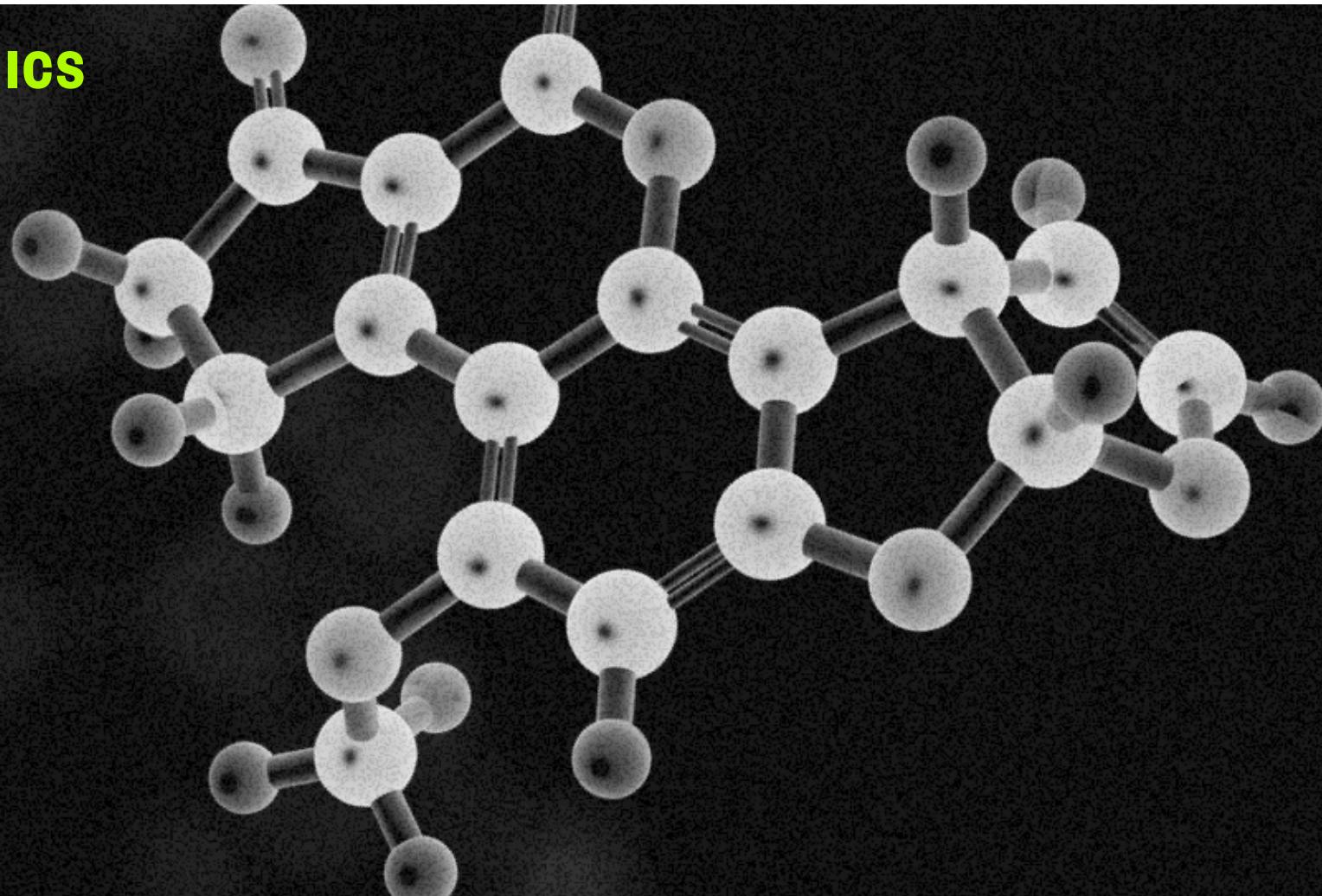


METABOLOMICS



Let's get hands dirty!

JACOB AGERBO RASMUSSEN

WHAT ARE WE GOING TO DO?

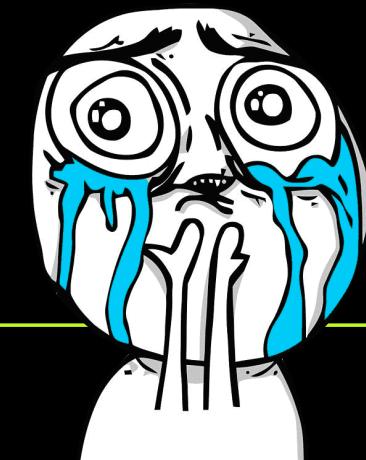
Mass preprocessing (very snappy)

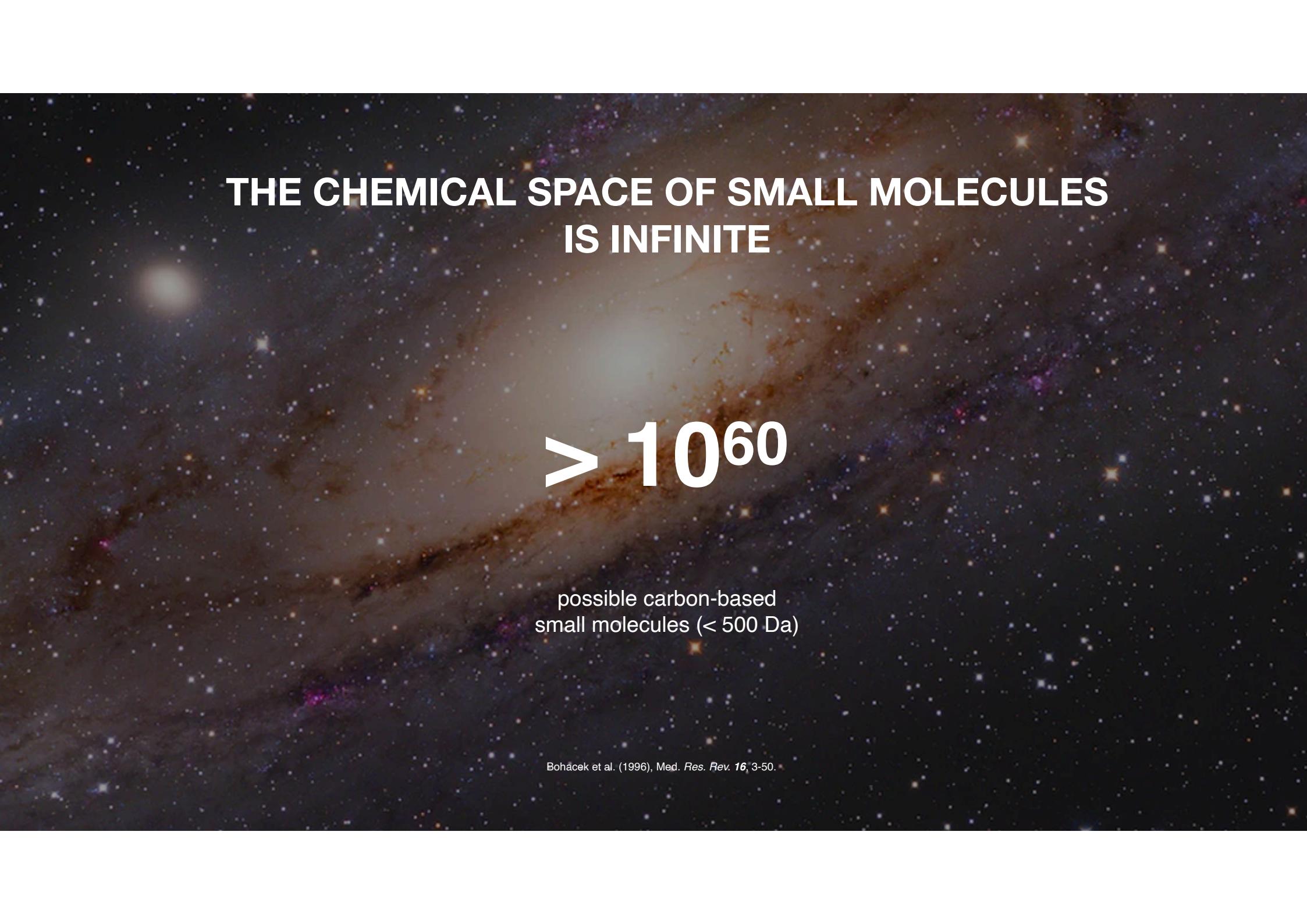


In silico Classifications



Multivariate Analysis





THE CHEMICAL SPACE OF SMALL MOLECULES IS INFINITE

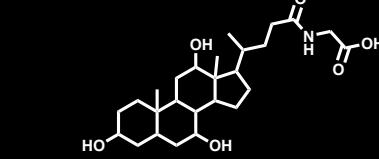
> 10⁶⁰

possible carbon-based
small molecules (< 500 Da)

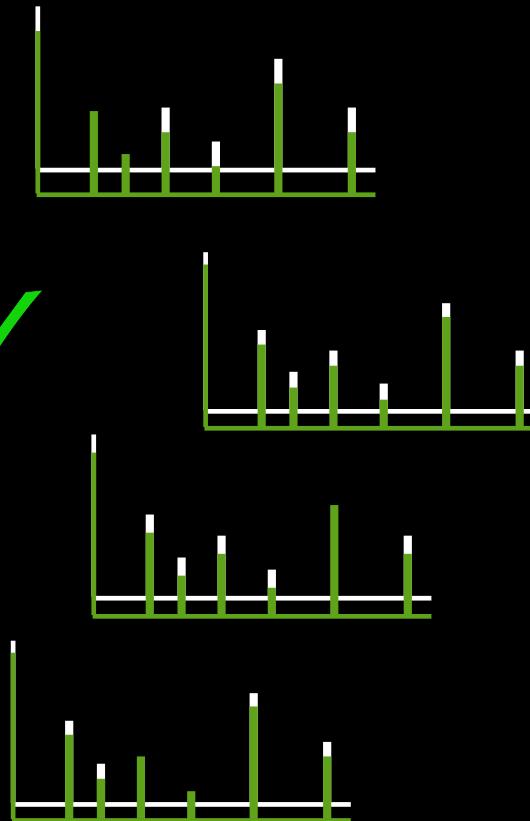
SPECTRUM LIBRARY MATCH

e.g. **BLAST** search

```
ATTTTACGTTAGCATCAGGTAGACT
TAAGGAGTCCGGTGTAGCCAAGGAC
ACTCCCAGGGAAATACGGGTGCGTG
TAAGGAGTCCGGTGTAGCCAAGGAC
CAAACGTTAATTCTCGGGTGTGTTG
TAAGGAGTCCGGTGTAGCCAAGGAC
CTCTTGAGATGGAGGAATATGTTTC
TAAGGAGTCCGGTGTAGCCAAGGAC
AGGTCTGGCTACGGAATAGATTGCA
TAAGGAGTCCGGTGTAGCCAAGGAC
TAAGGAGTCCGGTGTAGCCAAGGAC
TAAGGAGTCCGGTGTAGCCAAGGAC
GGTAGTAACCCTGGCTTCGGGTA
TAAGGAGTCCGGTGTAGCCAAGGAC
GGAAGGAGACACAGATAGACACGGT
TAAGGAGTCCGGTGTAGCCAAGGAC
TAACGACTAGGTCAAATAGAGTGCT
TAAGGAGTCCGGTGTAGCCAAGGAC
GTCTGGCTTAGAATTCACTATAGT
TAAGGAGTCCGGTGTAGCCAAGGAC
```



MS2 spectrum search



Adapted from Daniel Petras, Dorrestein Lab

Credits to Madeleine Ernst

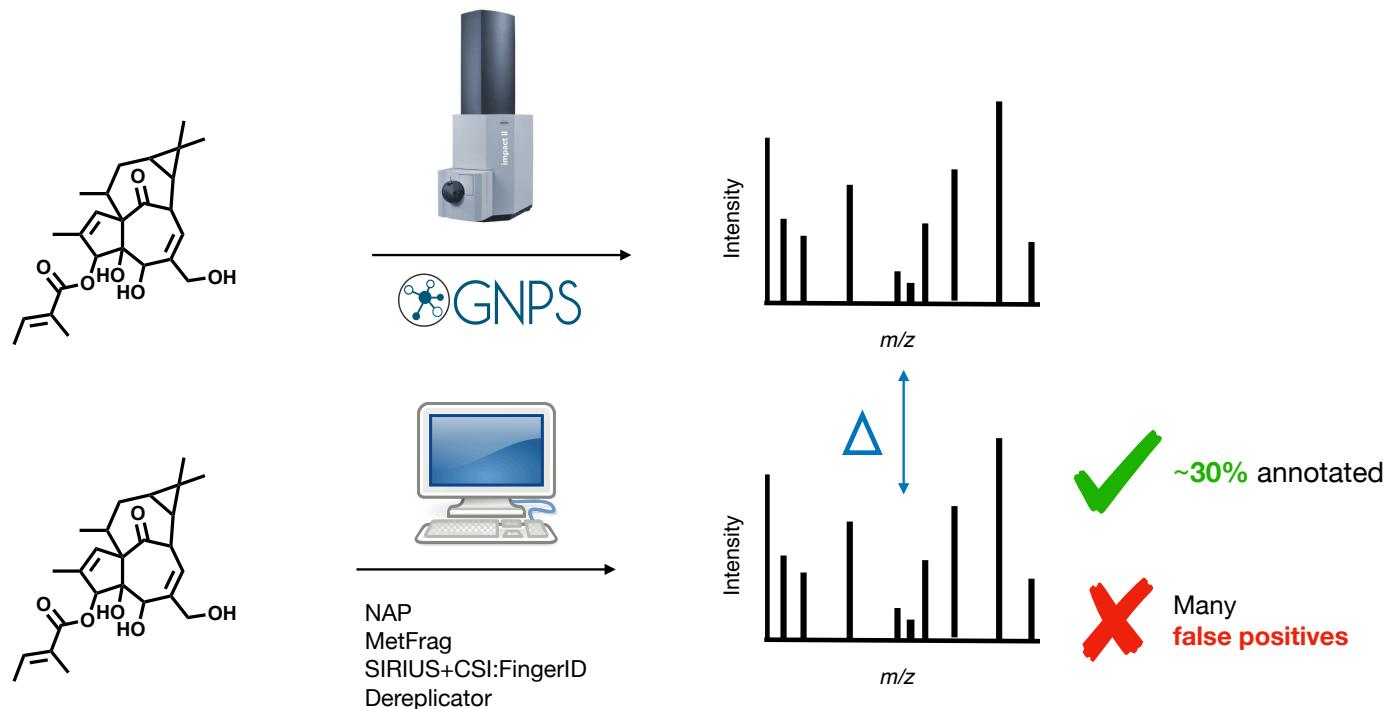
WHAT IF THE STRUCTURE IS NOT IN A DATABASE?

Only ~ 2% of the data that are collected in a typical LC-MS/MS experiment can be matched to known molecules

Aksenov et al. (2017), Nature Reviews Chemistry 1, 0054; da Silva et al. (2015), PNAS 112, 12549-12550.

Credits to Madeleine Ernst

SPECTRAL LIBRARY MATCHING VS *IN SILICO* ANNOTATION



da Silva et al. (2018) *PLoS Comput. Biol.* **14**, e1006089; Dürkop et al. (2015) *Proc. Natl. Acad. Sci. U S A* **112**, 12580-12585;
Mohimani et al. (2017) *Nat. Chem. Biol.* **13**, 30-37.

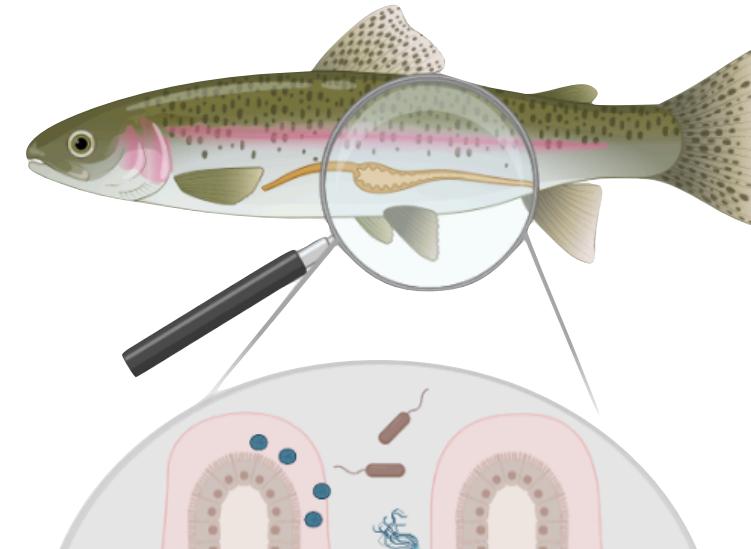
HANDS-ON!

Please go to: <https://github.com/EBI-Metagenomics/holofood-course>

Go to Sessions

And find the Metabolomics session

Log in to your VMs



Microbiome

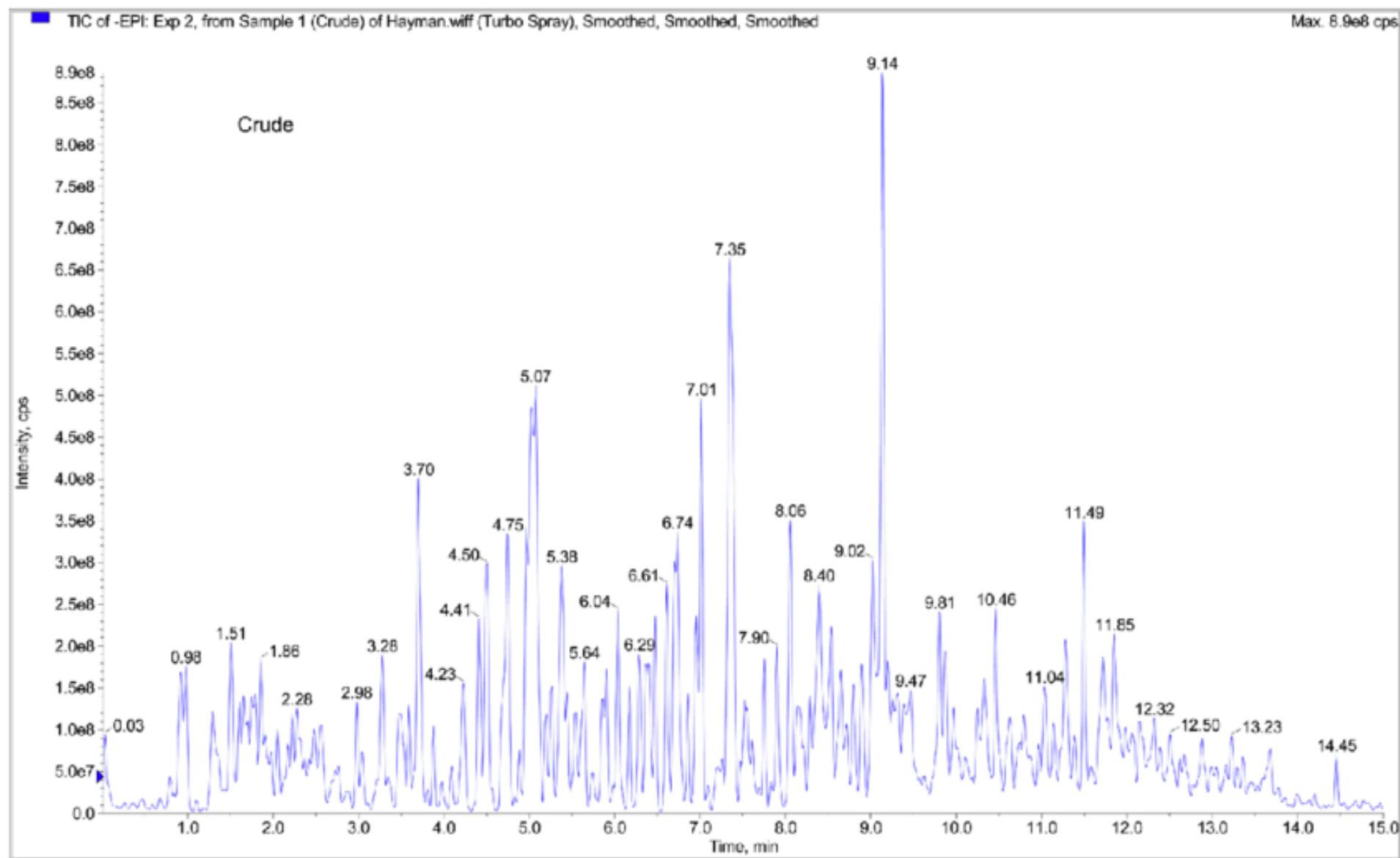
[Home](#) [About](#) [Articles](#) [Sections](#) [Collections](#) [Submission Guidelines](#)

Research | [Open Access](#) | Published: 30 January 2022

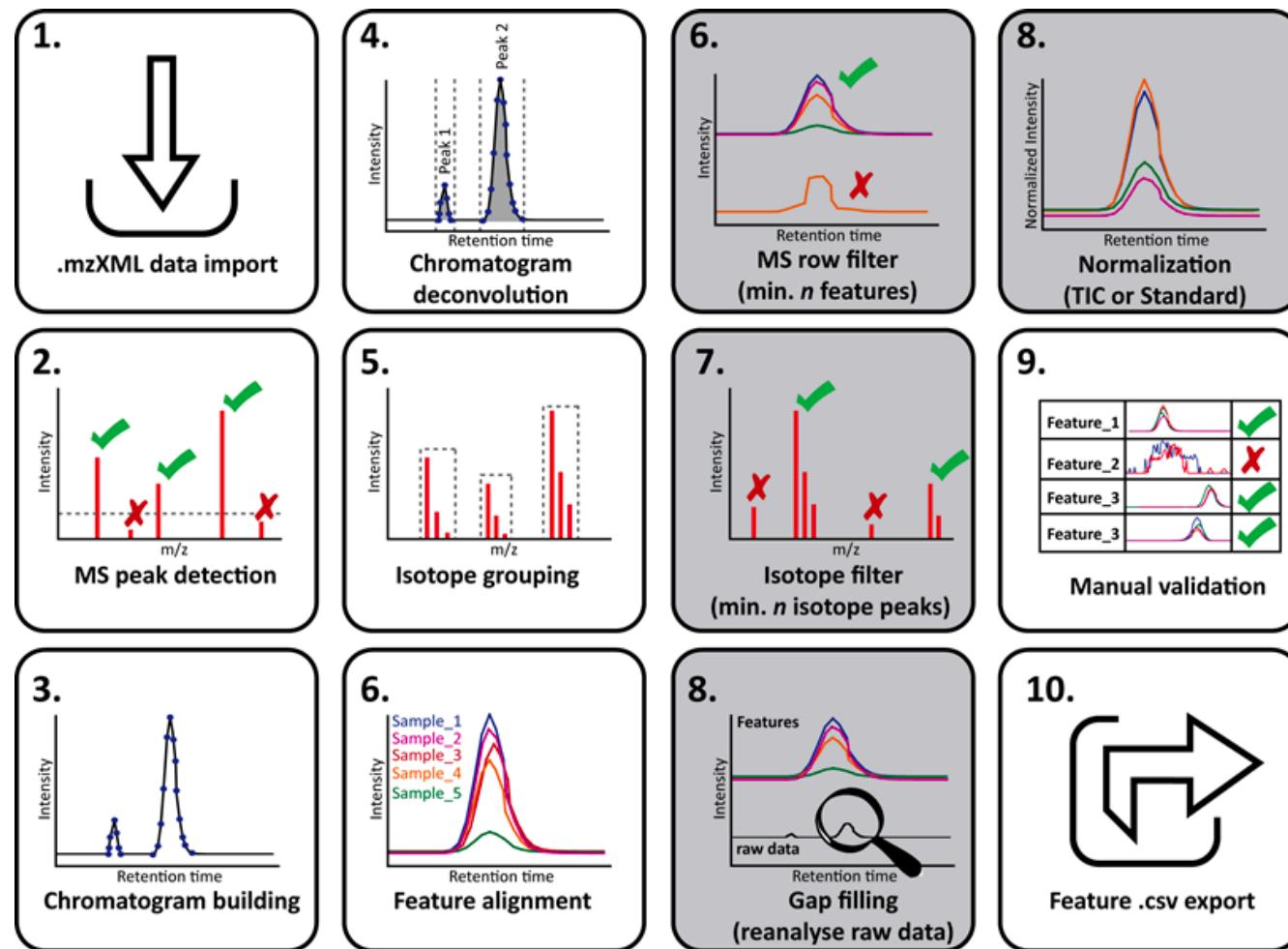
A multi-omics approach unravels metagenomic and metabolic alterations of a probiotic and symbiotic additive in rainbow trout (*Oncorhynchus mykiss*)

Jacob Agerbo Rasmussen , Kasper Rømer Villumsen, Madeleine Ernst, Martin Hansen, Torunn Forberg, Shyam Gopalakrishnan, M. Thomas P. Gilbert, Anders Miki Bojesen, Karsten Kristiansen & Morten Tønsberg Limborg

Rasmussen, J.A., et al. A multi-omics approach unravels metagenomic and metabolic alterations of a probiotic and symbiotic additive in rainbow trout (*Oncorhynchus mykiss*). *Microbiome* 10, 21 (2022). <https://doi.org/10.1186/s40168-021-01221-8>



MZmine 3



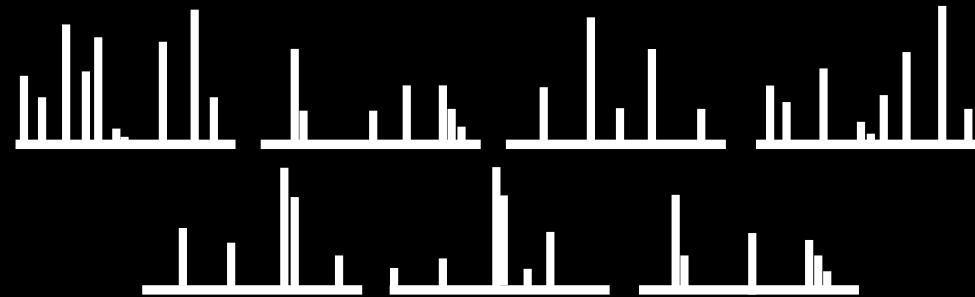
9



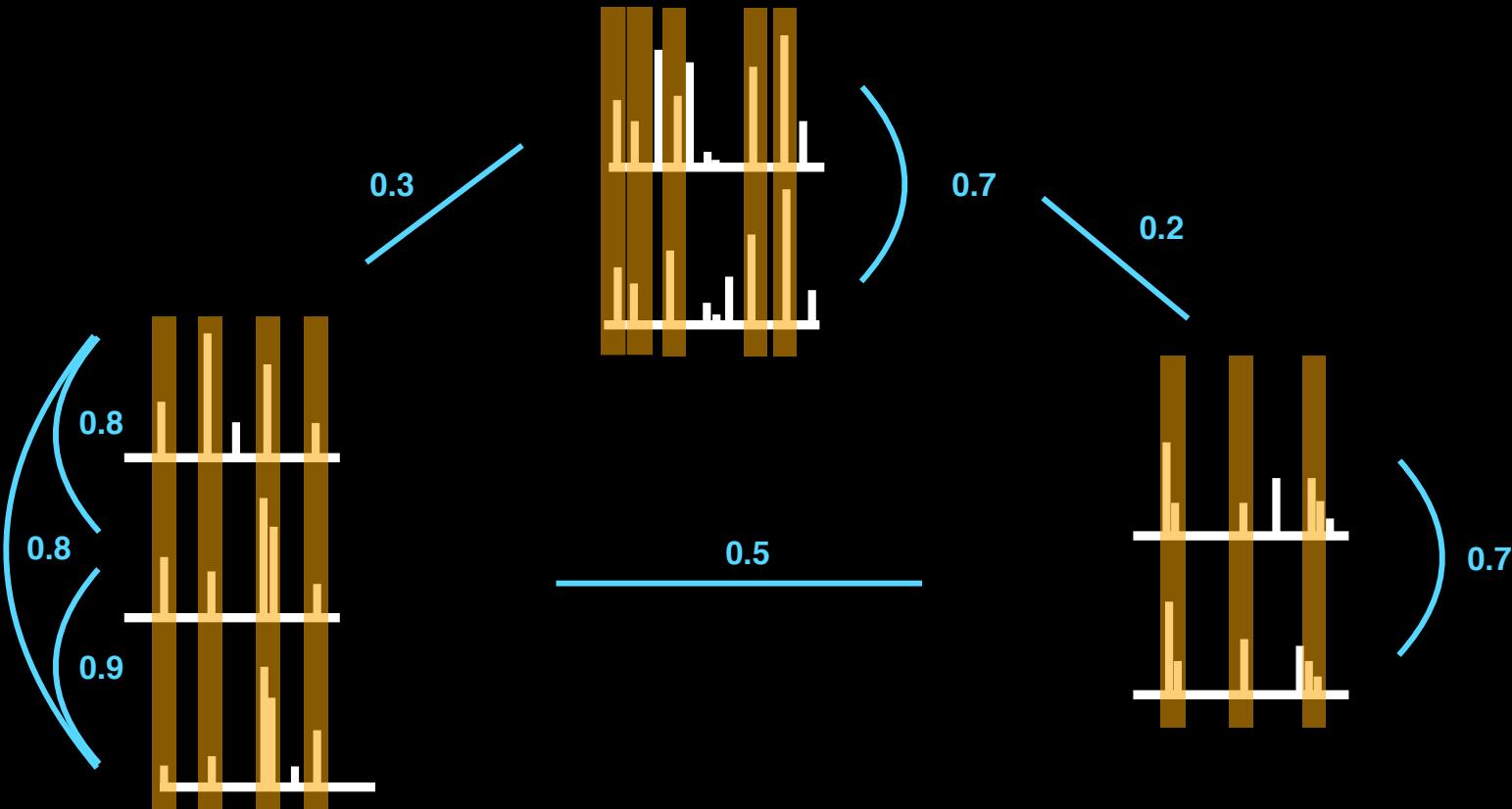
IN SILICO ANNOTATION



Experimental MS/MS spectra



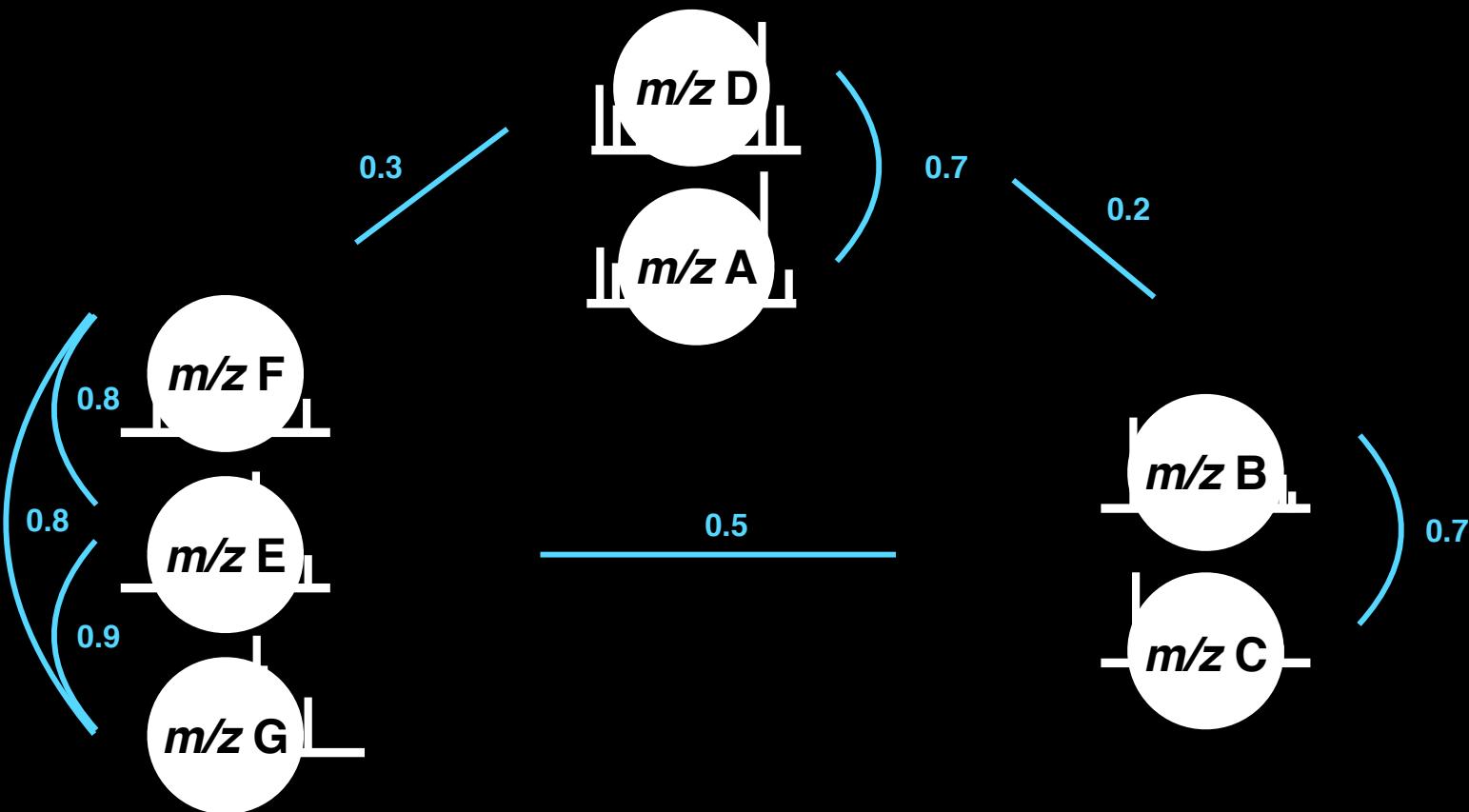
Experimental MS/MS spectra



Cosine score: 0 (no similarity) - 1 (identical)

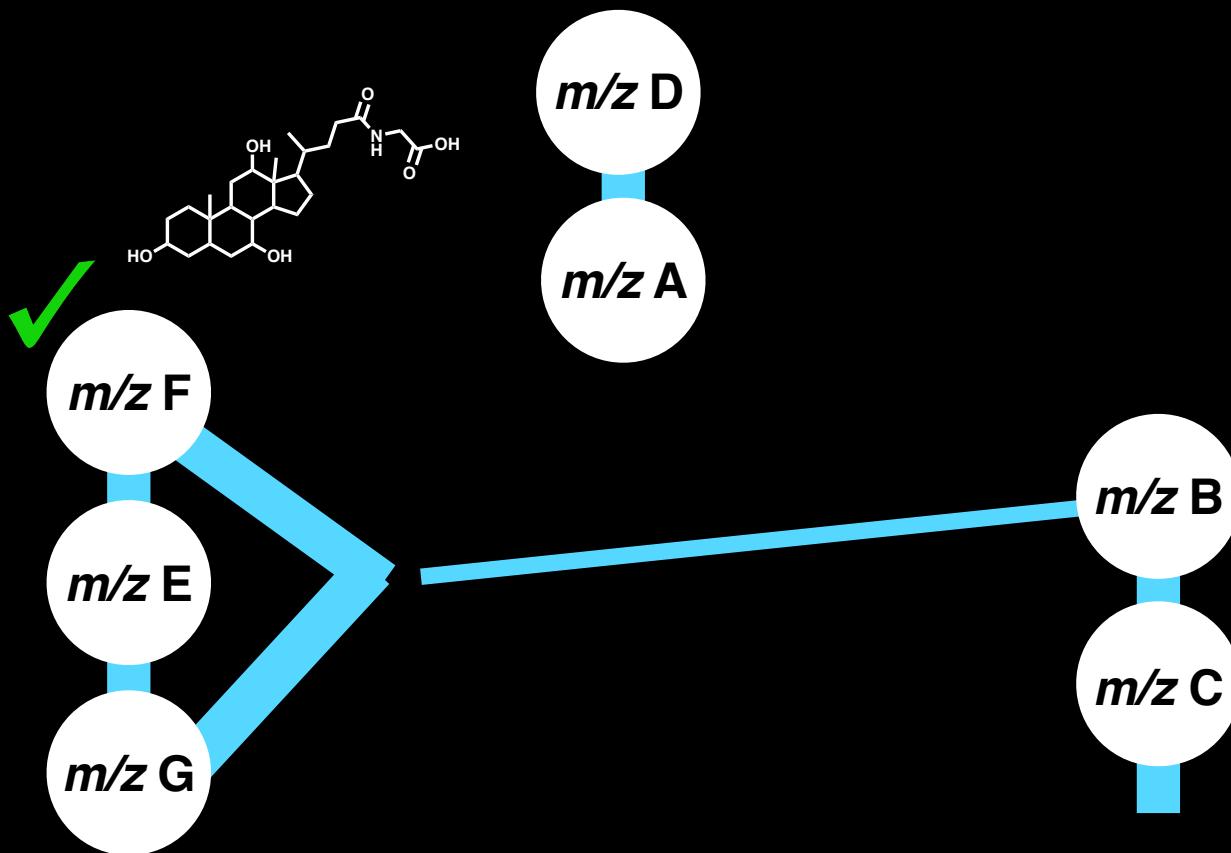
Watrous et al. (2012), PNAS 109, E1743-E1752;
Adapted from Daniel Petras, Dorrestein Lab

Experimental MS/MS spectra



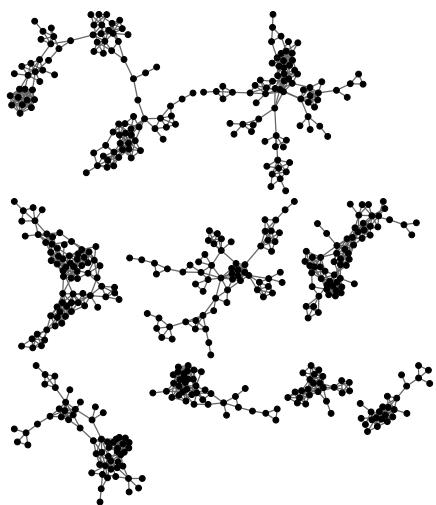
Watrous et al. (2012), PNAS 109, E1743-E1752;
Adapted from Daniel Petras, Dorrestein Lab

Experimental MS/MS spectra



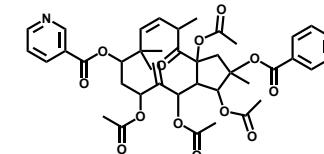
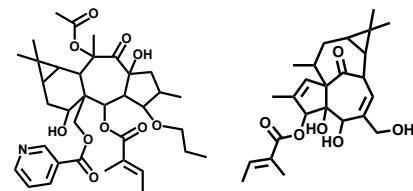
Watrous et al. (2012), PNAS 109, E1743-E1752;
Adapted from Daniel Petras, Dorrestein Lab

CHEMICAL CLASS ANNOTATION



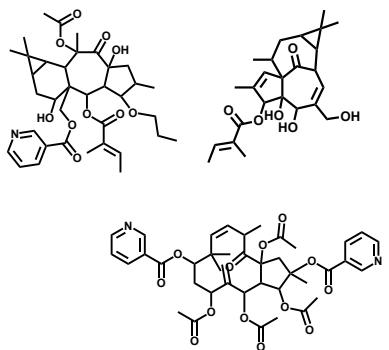
In silico structure prediction
(NAP, SIRIUS+CSI:FingerID, Dereplicator...)

→
Spectral matches
to GNPS libraries



da Silva et al. (2018) *PLoS Comput. Biol.* **14**, e1006089; Dührkop et al. (2015) *Proc. Natl. Acad. Sci. U S A* **112**, 12580-12585;
Mohimani et al. (2017) *Nat. Chem. Biol.* **13**, 30-37.

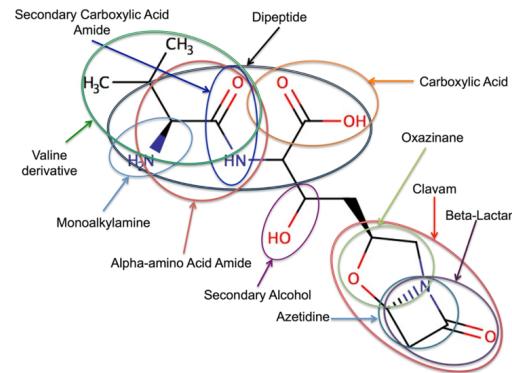
CHEMICAL CLASS ANNOTATION



Automated chemical classification



ClassyFire or CANOPUS



Kingdom: Organic compounds

Superclass: Lipids and lipid-like molecules

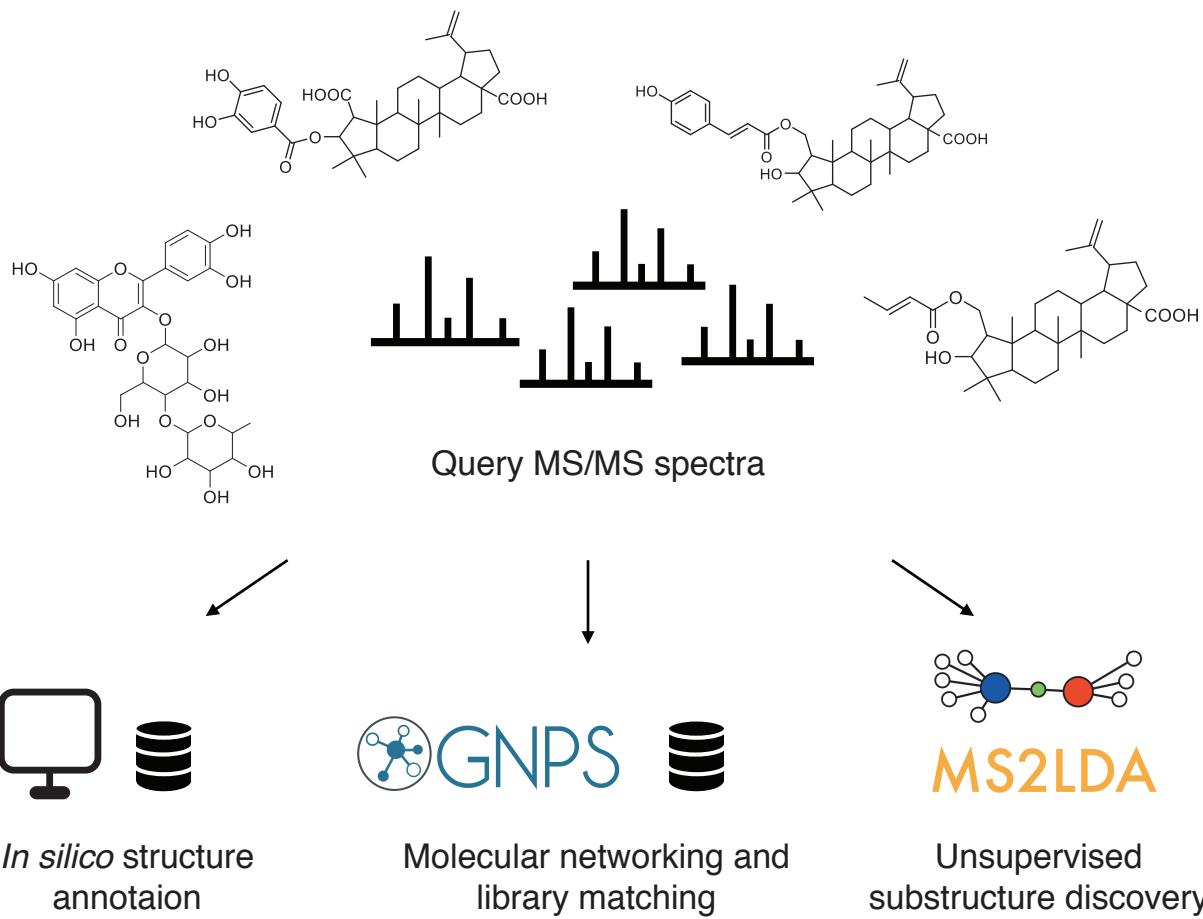
Class: Prenol lipids

Subclass: Diterpenoids

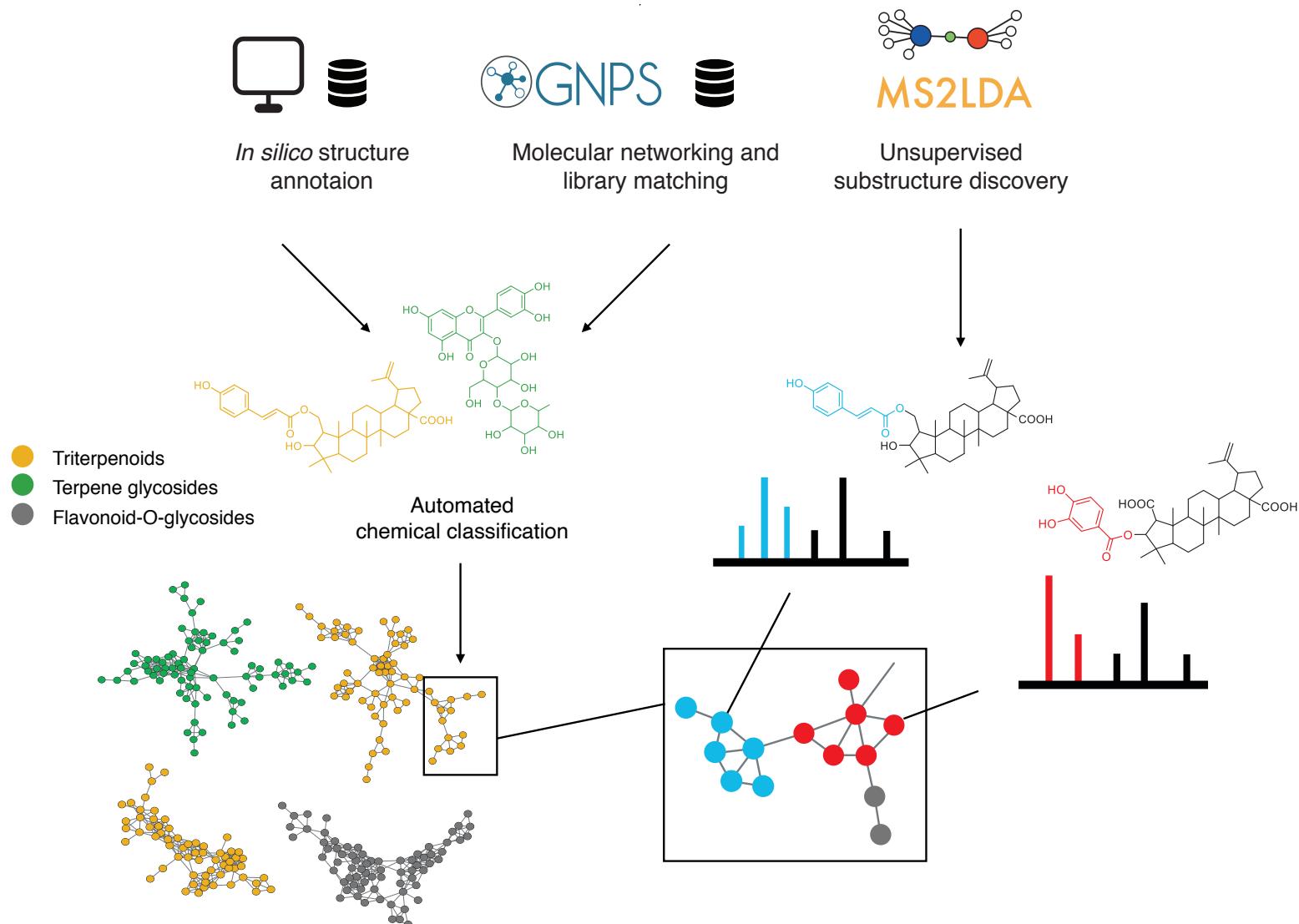
...

Feunang et al. (2016), *J. Cheminform.* **8**, 61.

MOLNETENHANCER

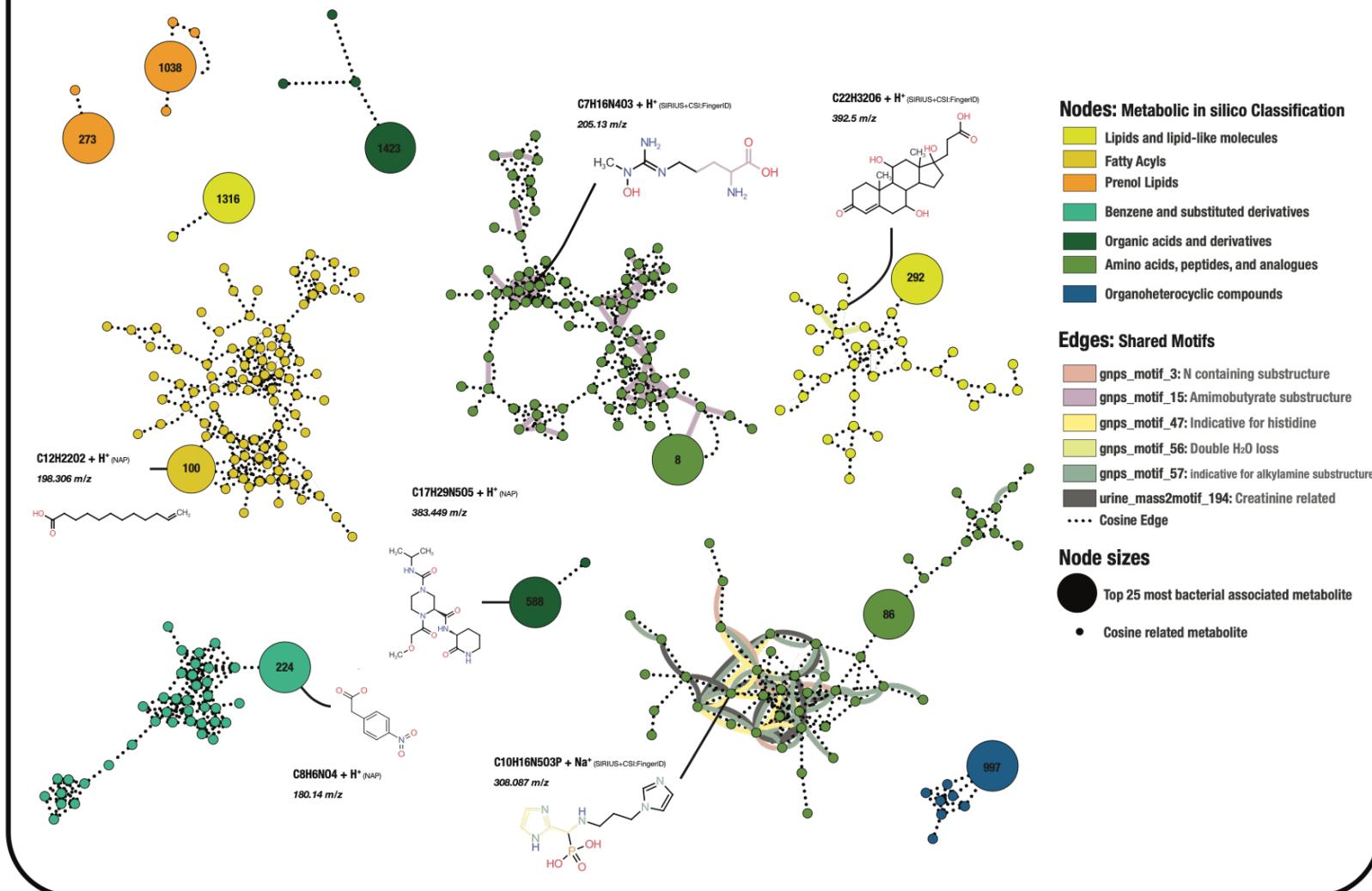


Ernst et al. (2019). <https://www.biorxiv.org/content/10.1101/654459v1>



Ernst et al. (2019). <https://www.biorxiv.org/content/10.1101/654459v1>

Deciphering unknown metabolites associated with gut related bacteria



MULTIVARIATE ANALYSIS

(CONSIDERATIONS)

Axis 2 (4.599 %)

- Bacteria
- Corals
- Environment
- Food
- Human
- Mouse
- Plants
- Standards
- Others

Axis 1 (6.744 %)

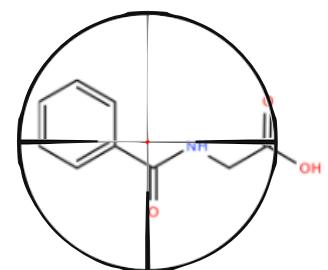
Axis 3 (4.143 %)

MULTIVARIATE ANALYSIS

(CONSIDERATIONS)

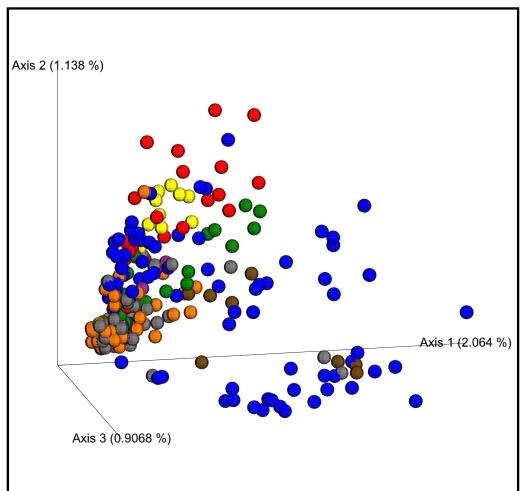
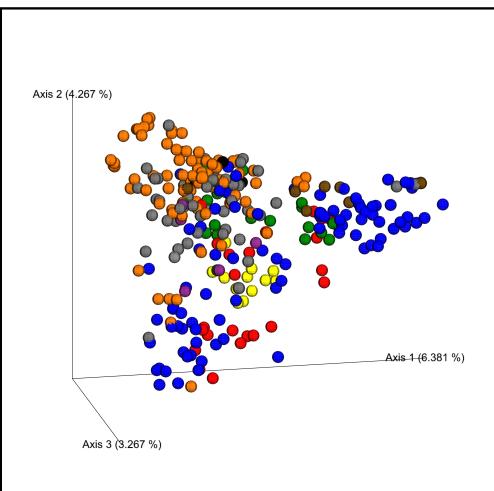
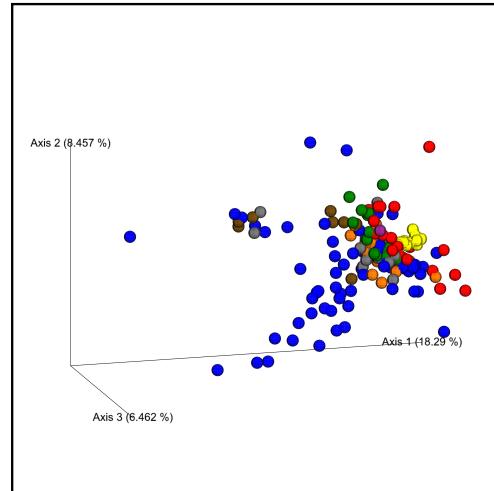
DON'T INVESTIGATE ALL
INVESTIGATE THE INTERESTING

NARROW DOWN THE NO. METABOLITES BEFORE
NETWORK ANALYSIS AND CURATION OF CLASSIFICATION

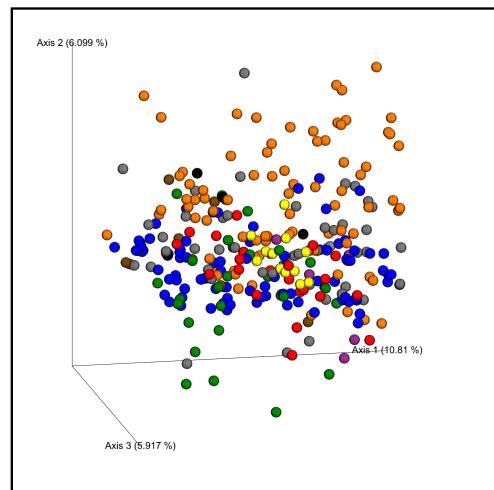
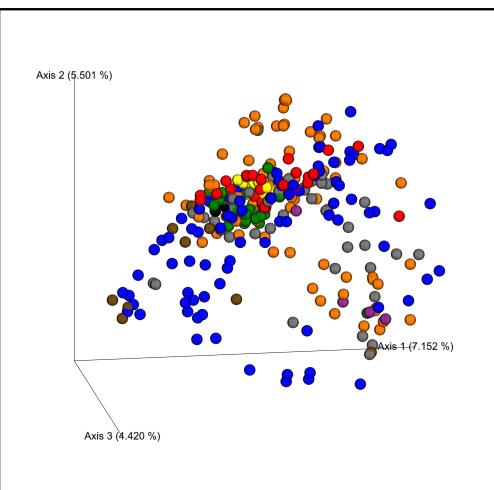
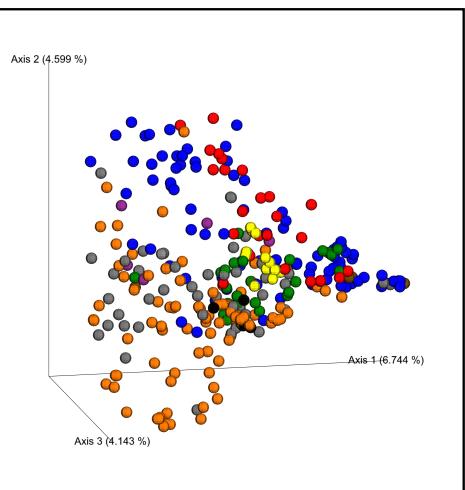


A dense tropical forest scene with sunlight filtering through leaves.

ARE WE USING
APPROPRIATE
DISTANCE METRICS?

KULSINSKI**JACCARD****CANBERRA**

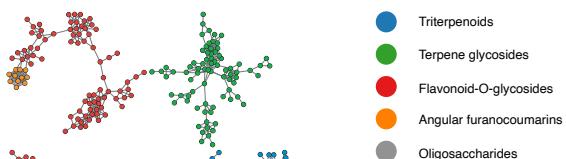
- Bacteria
- Corals
- Environment
- Food
- Human
- Mouse
- Plants
- Standards
- Others

**BRAY-CURTIS****EUCLIDEAN****GOWER**

UNIFRAC INSPIRED METRICS FOR METABOLOMICS DATA

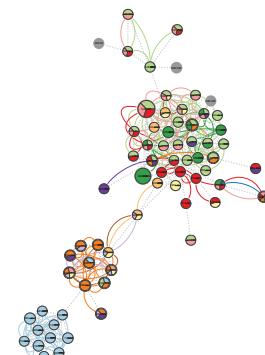
Supervised

CLASSYTREE UNIFRAC

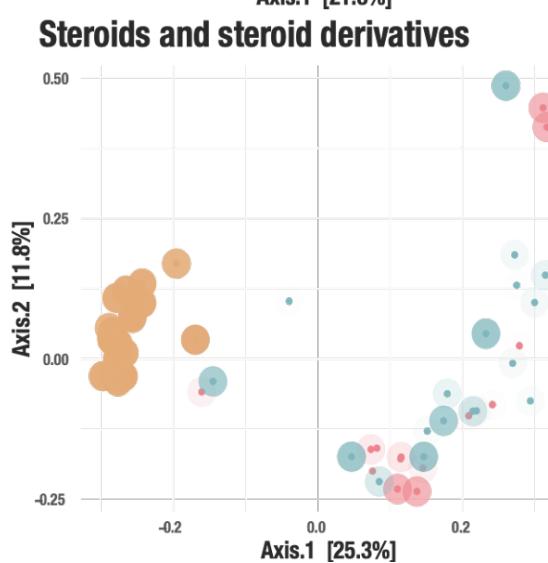
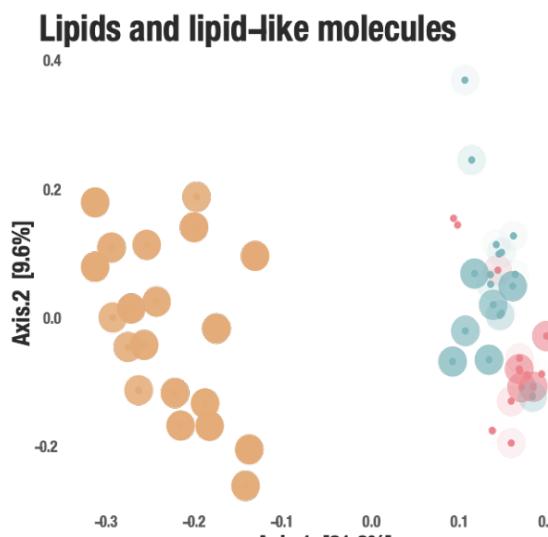
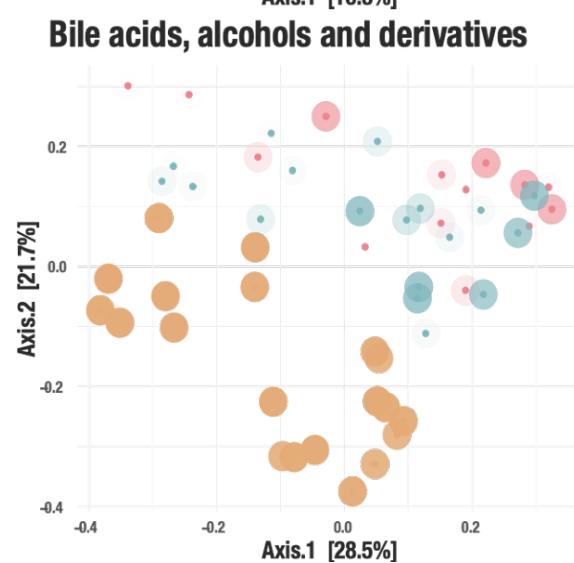
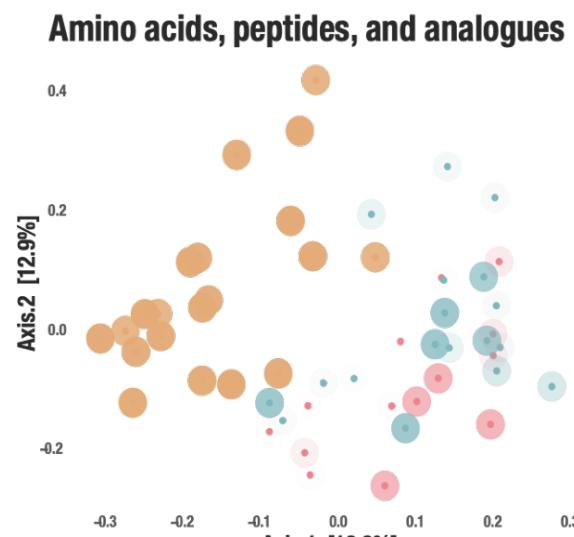


Unsupervised

MOTIFTREE UNIFRAC



Ernst, M et al., *in preparation*; Lozupone and Knight (2005), *Applied and environmental microbiology* **71**, 8228-8235.



Feed

- Control
- Probiotics
- Synbiotics

Mycoplasma Abundance

1.0
0.0

ACKNOWLEDGEMENT

Credits to Madeleine Ernst

QUESTIONS

jacob.rasmussen@sund.ku.dk