Application of High Resolution UPLC-MS-MS Towards the Prioritization of Marine Bacteria-Derived Crude Extracts for Natural Product Drug Discovery

Megan S. Rothenberg, Jake L. Belli, Christine M. Theodore, Ph.D.
The University of Tampa

Introduction

Marine natural products (NP) are a prolific source of pharmaceutical drug leads, requiring an efficient extract prioritization method to facilitate further biological testing.

Challenges –
- Laborious purification processes for efficient HTS.
- Inefficient dereplication and structure elucidation.
- Limited availability of material for bioactive testing.

Spectrometric analysis –
- Comparison of MS/MS spectral data denotes fragmentation similarities displayed in molecular networks.
- Useful for dereplication, structure elucidation, and extract prioritization.

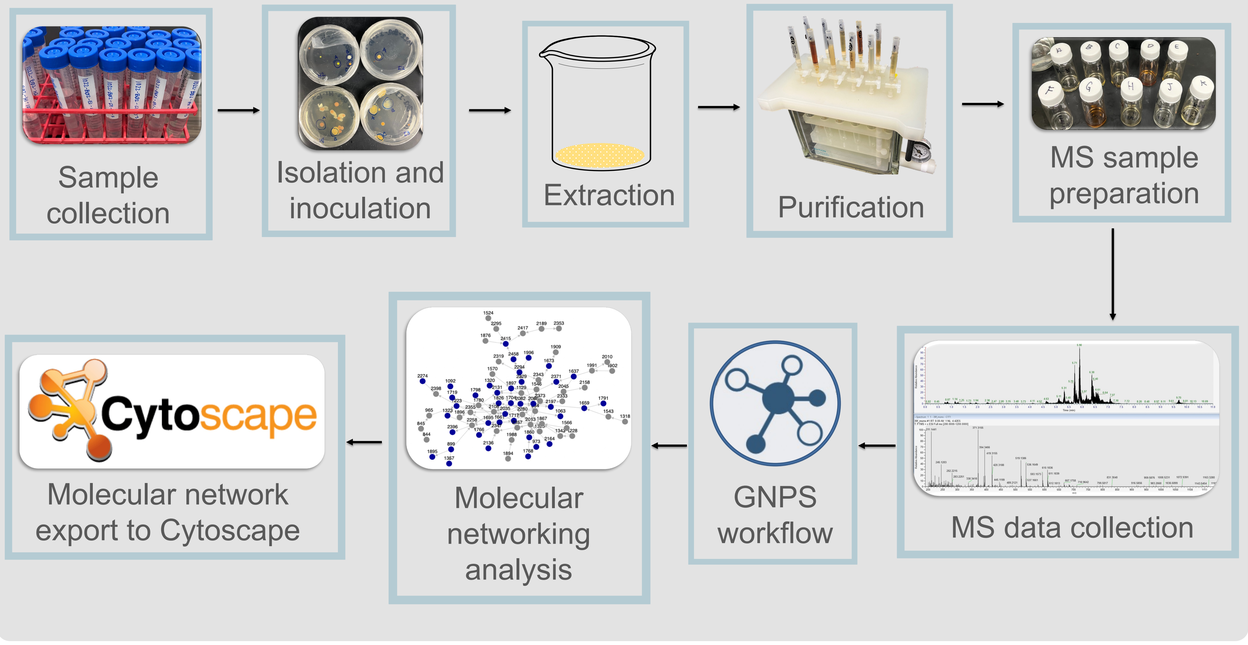
Global Natural Products Social (GNPS) Molecular Networking –
- Develops molecular networks from stored spectral library data (MassIVE) and metadata.
- Offers advanced spectral search options (MASST).

Project goal –
- Utilize molecular networks to characterize extracts and prioritize potential bioactive leads.

Molecular Networking

- Visual display of spectral relatedness
- Detects sets of spectra from related molecules with structural similarities
- Similar spectra a clustered in nodes connected by edges based on cosine score

Methods



Parameters

Vanquish UPLC

Flow rate	0.250 [ml/min]
Run time	11.000 [min]
Pump pressure limits	lower: 10 [bar] upper: 600 [bar]
Maximum flow accel/decel	up/down: 6.00 [ml/min²]
Speed parameters	draw/dispense: 5.000 [µl/s]
Column (C18 Kinetex®)	length – 100 [mm] diameter – 2.1 [mm] pore size – 100 [Å] particle size – 1.7 [µm]
Temperature	sampler – 20.0 [°C] column – 40.0 [°C]

Orbitrap Exploris 120

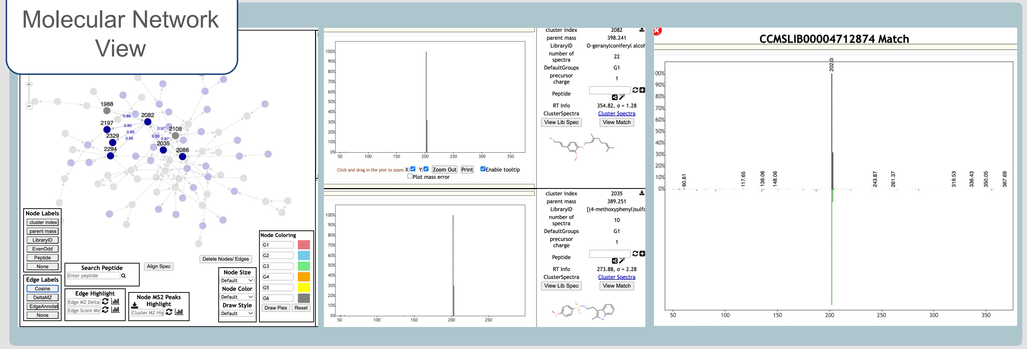
Application mode	small molecule
Data type	centroid
Polarity	positive
Scan range (m/z)	100-1200
Orbitrap resolution	120000
Infusion mode	liquid chromatography
Internal mass calibration	EASY-IC™
RF lens	70 [%]
Injection mode	auto

Future Work

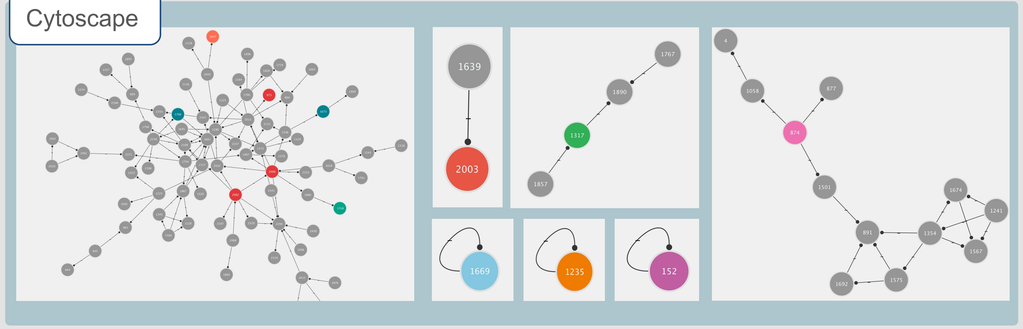
- Extrapolation of molecular networks allows for extract characterization and prioritization.
- Advanced spectral searches (MASST) and match analysis will aid in dereplication and structure elucidation.
- Mapping of metadata onto molecular networks will continue extract characterization.
- Prioritized extracts will go through optimization of growth conditions and biological testing.
- Large scale grow-ups can be done using a bioreactor.

Results

Molecular Network View



Cytoscape



Prioritized extract	Clusters	Compounds	Cos	Structures
A	152	Dodecanedioic acid (**2**)	0.89	(**1**)
	1637	Dioctyl phthalate (**3**)	0.88	
	2086	O-geranylconiferyl alcohol (**1**)	0.90	
	880	Mucic acid (**4**)	0.88	(**2**)
D	1235	O-geranylconiferyl alcohol (**1**)	0.92	
	1637	Dioctyl phthalate (**3**)	0.88	(**3**)
	2003	11-deoxycortisol^ (**5**)	0.71	
E	152	Dodecanedioic acid (**2**)	0.89	
	1637	Dioctyl phthalate (**3**)	0.88	(**4**)
	973, 2086, 2082	O-geranylconiferyl alcohol (**1**)	0.92	
	2212	1-[(3,4-dimethoxyphenyl)methyl]-6,7-dimethoxyisoquinoline^ (**6**)	0.80	(**5**)^
K	152	Dodecanedioic acid (**2**)	0.88	
	2082	O-geranylconiferyl alcohol (**1**)	0.92	
	1673	1-[(3,4-dimethoxyphenyl)methyl]-6,7-dimethoxyisoquinoline^ (**6**)	0.82	(**6**)^ (**7**)^
37v	1768	3’,4’,7’-trihydroxyflavanone^ (**7**)	0.79	
	973, 2086	O-geranylconiferyl alcohol (**1**)	0.91	(**8**) (**9**)
39f	1317	Papaverine (**8**)	0.88	
	1669	Pyochelin (**9**)	0.91	
	1766	1-[(3,4-dimethoxyphenyl)methyl]-6,7-dimethoxyisoquinoline^ (**6**)	0.92	(**10**)
	874	2-heptylquinolin-4(1H)-one (**10**)	0.88	

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