Metabolite discovery through global annotation of untargeted metabolomics data

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Supplementary table 1. List of biochemical atom differences

Type	Formula / Atom difference	Mass difference	RDBE[1] difference	Allowed propagation direction[2]
Deamination	O1N-1H-1	0.98402	0	0
Transamination	N1H3O-1	1.03163	-1	0
Hydrogenation	H2	2.01565	-1	0
Methylation	C1H2	14.01565	0	0
Amination	N1H1	15.01090	0	0
Hydroxylation	O1	15.99491	0	0
Amination	N1H3	17.02655	-1	0
Hydration	H2O1	18.01056	-1	0
Formylation	C1O1	27.99491	1	0
Beta oxidation	C2H4	28.03130	0	0
Deamination	C1H2O1	30.01056	0	0
Thiolation	S1	31.97207	0	0
Sulfurization	H2S1	33.98772	-1	0
Acetylation	C2H2O1	42.01056	1	0
Carboxylation	C1O2	43.98983	1	0
Isoprenylation	C5H8	68.06260	1	1
Sulfurylatoin	S1O3	79.95681	0	0
Phosphorylation	H1P1O3	79.96633	0	0
Hexose	C6H10O5	162.05282	1	1
Uronate	C6H8O6	176.03209	2	1
Palmitoylation	C16H30O1	238.22967	1	1
Sialic acid	C11H17N1O8	291.09542	3	1
AMP	C10H12N5O6P1	329.05252	7	1
CDP	C9H13N3O10P2	385.00762	5	1
ADP-ribosylation	C15H21N5O13P2	541.06111	8	1

1. RDBE stands for ring and double bond equivalence.
2. Allowed propagation direction: “1” means only forward propagation is allowed, i.e. adding the indicated atom differences to the parent formula; “-1” means reverse only, i.e. subtracting the indicated atom differences from the parent formula, and “0” means propagation is allowed for both directions.

Supplementary table 2. List of abiotic atom differences

Type	Formula / Atom difference	Mass difference	RDBE difference	Allowed propagation direction
Isotope	[10]B-1B1	0.99637	0	-1
Isotope	[15]N1N-1	0.99703	0	1
Isotope	[29]Si-1[30]Si1	0.99728	0	1
Isotope	[29]Si1Si-1	0.99957	0	1
Isotope	[53]Cr1Cr-1	1.00014	0	1
Isotope	[13]C1C-1	1.00335	0	1
Isotope	[2]H1H-1	1.00628	0	1
Isotope	[34]S1S-1	1.99580	0	1
Isotope	[30]Si1Si-1	1.99684	0	1
Isotope	[37]Cl1Cl-1	1.99705	0	1
Isotope	[41]K1K-1	1.99812	0	1
Isotope	[18]O1O-1	2.00425	0	1
Isotope	[44]Ca1Ca-1	3.99289	0	1
Isotope	[60]Ni1Ni-1	1.99544	0	1
Isotope	[62]Ni1Ni-1	3.99300	0	1
Adduct	H-1Na1	21.98194	0	1
Adduct	Cl1H1	35.97668	0	1
Adduct	H-1K1	37.95588	0	1
Adduct	H-2Ni1	55.91969	0	1
Adduct	Ca1H-2	37.94694	0	1
Adduct	C1H2O2	46.00548	0	1
Adduct	C1H1Na1O2	67.98742	0	1
Adduct	C1H1K1O2	83.96136	0	1
Adduct	C2H4O2	60.02113	0	1
Adduct	C2H3Na1O2	82.00307	0	1
Adduct	C2H3K1O2	97.97701	0	1
Adduct	C2H2Ni1O2	115.94082	0	1
Adduct	C2Ca1H2O2	97.96807	0	1
Adduct	H2O4S1	97.96738	0	1

Type	Formula / Atom difference	Mass difference	RDBE difference	Allowed propagation direction
Adduct	H1Na1O4S1	119.94932	0	1
Adduct	H1K1O4S1	135.92326	0	1
Adduct	H1N1O3	62.99564	0	1
Adduct	N1Na1O3	84.97759	0	1
Adduct	H2C1O3	62.00039	0	1
Adduct	Na1H1C1O3	83.98234	0	1
Adduct	K1H1C1O3	99.95628	0	1
Adduct	H3O4P1	97.97690	0	1
Adduct	H2Na1O4P1	119.95884	0	1
Adduct	H2K1O4P1	135.93278	0	1
Adduct	Cr1O3	99.92525	0	1
Adduct	H4O4Si1	95.98789	0	1
Adduct	H3N1	17.02655	0	1
Adduct	H-2Na2	43.96389	0	1
Adduct	H-2K2	75.91176	0	1
Adduct	C1H1N1	27.01090	0	1
Adduct	C1H4O1	32.02621	0	1
Adduct	H6O8P2	195.95379	0	1
Adduct	B1H-3	7.98583	2	1
Adduct	B1H-1O1	25.99640	1	1
Adduct	H2O3Si1	77.97732	0	1
Adduct	H1Na1O1	39.99251	0	1
Adduct	H1K1O1	55.96645	0	1
Adduct	C2H3N1	41.02655	0	1
Adduct	C3H8O3Si1	120.02427	0	1
Fragment	C1O2	43.98983	1	-1
Fragment	C1H2O1	30.01056	0	-1
Fragment	H2O1	18.01056	-1	-1
Fragment	N1H3	17.02655	-1	-1
Radical	H	1.00783	-0.5	-1

Supplementary table 3. Examples of nickel related peaks

peak_id	medMz	medRt	log10_inten	formula	Δppm	annotation
4	89.0476	13.01	8.65	C3H7N1O2	0.92	C3H7N1O2
1194	294.0362	13.01	5.37	C8H16N2Ni1O6	0.01	C3H7N1O2 * 2 + C2H2Ni1O2
1952	296.0316	13.01	4.98	[60]Ni1C8H16N2O6	0.16	C3H7N1O2 * 2 + C2H2Ni1O2 + [60]Ni1Ni-1*
2723	234.0149	13	4.67	C6H12N2Ni1O4	0.74	C3H7N1O2 * 2 + H-2Ni1
2269	265.0095	13.07	4.92	C7H13N1Ni1O6	0.58	C3H7N1O2 + C2H4O2 + C2H2Ni1O2
3869	267.005	13.04	4.36	[60]Ni1C7H13N1O6	0.36	C3H7N1O2 + C2H4O2 + C2H2Ni1O2 + [60]Ni1Ni-1
855	270.0961	13.59	5.62	C10H14N4O5	1.27	C10H14N4O5
2005	358.0423	13.5	4.97	C11H16N4Ni1O6	0.14	C10H14N4O5 + C1H4O1 + H-2Ni1
2961	360.0377	13.51	4.39	[60]Ni1C11H16N4O6	0.26	C10H14N4O5 + C1H4O1 + H-2Ni1 + [60]Ni1Ni-1
125	222.0673	14.18	6.82	C7H14N2O4S1	0.68	C7H14N2O4S1
2406	338.0083	14.29	4.95	C9H16N2Ni1O6S1	-0.08	C7H14N2O4S1 + C2H2Ni1O2
31	117.079	11.21	7.61	C5H11N1O2	0.02	C5H11N1O2
3450	293.041	11.24	4.54	C9H17N1Ni1O6	-0.16	C5H11N1O2 + C2H4O2 + C2H2Ni1O2
4664	146.069	13.39	8.28	C5H10N2O3	1.13	C5H10N2O3
3287	262.0098	13.4	4.53	C7H12N2Ni1O5	0.72	C5H10N2O3 + C2H2Ni1O2
3534	322.0311	13.37	4.38	C9H16N2Ni1O7	0.05	C5H10N2O3 + C2H2Ni1O2 + C2H4O2
92	612.1521	14.19	6.98	C20H32N6O12S2	-0.19	C20H32N6O12S2
2440	668.0718	14.2	4.87	C20H30N6Ni1O12S2	-0.18	C20H32N6O12S2 + H-2Ni1
3528	670.0671	14.21	4.54	[60]Ni1C20H30N6O12S2	0.03	C20H32N6O12S2 + H-2Ni1 + [60]Ni1Ni-1
22	132.0898	16.24	7.34	C5H12N2O2	0.77	C5H12N2O2
4942	308.052	16.26	4.1	C9H18N2Ni1O6	-0.48	C5H12N2O2 + C2H4O2 + C2H2Ni1O2

* “[60]Ni1Ni-1” means adding an isotope ^{60}Ni and subtracting a regular Ni, representing the atom difference of nickel isotope. This representation aligns atom difference to mass difference.

Supplementary table 4. Search results of newly identified metabolites in compound databases

	HMDB		PubChem		METLIN		Sci-Finder	
	formula	structure	formula	structure	formula	structure	formula	structure
Thiamine + [C_2H_2O] ($C_{14}H_{18}N_4O_2S$)	X	X	√	X	X	X	√	√[2]
Thiamine + [C_2H_4O] ($C_{14}H_{20}N_4O_2S$)	X	X	√	X[1]	X	X	√	X
Thiamine + [$C_4H_6O_3$] ($C_{16}H_{22}N_4O_4S$)	X	X	√	X[1]	X	X	√	X
Thiamine + [C_4H_8O] ($C_{16}H_{24}N_4O_2S$)	X	X	√	X[1]	X	X	√	X
Glucosyl-taurine ($C_8H_{17}NO_8S$)	X	X	√	√[2]	X	X	√	√[2]

Note:
1. Pyrophosphate form of the metabolite exists.
2. Reported only as a synthetic chemical, not a metabolite or biological chemical.

Supplementary Table 5. Memory and run-time used in NetID.

	Yeast neg	Yeast pos	Liver neg	Liver pos
Total non-background peaks	5588	9833	8191	12128
Maximum memory used (GB)	4.7	13.3	6.8	12.1
Optimization time (min)	2.5	6.7	1.1	2.4
Total time (min)	24.3	101.3	31.8	94.4

Note: The maximum memory and run-time reported here is under default parameter setting in NetID.

Supplementary Note 1 – NetID scoring parameters

Scoring candidate node annotations

NetID scores every candidate node and edge annotation assigned in the candidate annotation step. The node scoring system aims to assign high scores to annotations that align observed ion peaks with known metabolites based on m/z, retention time, MS/MS, and/or isotope abundances.

Let the set of candidate annotations for node *u* be denoted as $\{a_1 \dots a_i \dots a_m\}$. For each node *u* and each of its candidate annotation a_i, let S(u, a_i) denotes the score of candidate annotation a_i for node *u*. Different scoring components for candidate node annotations are defined as below:

(a) $\mathrm{S}_{\mathrm{m/z}}(u, a_i)$ is negative when measured m/z differs from the calculated m/z of assigned molecular formula. A larger ppm difference between calculated formula m/z and measurement m/z results to lower scores. The default scale factor is -0.5. Let $a_{i,\mathrm{m/z}}$ be the calculated m/z of annotation a_i, and $u_{\mathrm{m/z}}$ be the measured m/z of node *u*, then

$$\mathrm{S}_{\mathrm{m/z}}(u, a_i) = -0.5 \times \left| u_{\mathrm{m/z}} - a_{i,\mathrm{m/z}} \right| / u_{\mathrm{m/z}} \times 10^6 \quad (1)$$

(b) $\mathrm{S}_{\mathrm{RT}}(u, a_i)$ is positive if the measured RT for the peak corresponding to node *u* matches to a known standard. A smaller difference between known and measured RT results in a higher score. Let $a_{i,\mathrm{RT}}$ be the known RT of annotation a_i, and u_{RT} be the measured RT of node *u*, then

$$\mathrm{S}_{\mathrm{RT}}(u, a_i) = 1 - \left| u_{\mathrm{RT}} - a_{i,\mathrm{RT}} \right|, \text{ if } \left| u_{\mathrm{RT}} - a_{i,\mathrm{RT}} \right| < 0.5 \text{ min}$$

$$\text{Otherwise, } \mathrm{S}_{\mathrm{RT}}(u, a_i) = 0 \quad (2)$$

(c) $\mathrm{S}_{\mathrm{MS2}}(u, a_i)$ is positive if the measured MS2 spectrum of node *u* matches the database MS2 spectrum of annotation a_i. A cosine similarity-based scoring function is used to score the MS2 spectra similarity[1,2]. The intensities of the fragment ions in the MS2 spectra are rescaled so that the highest fragment ion is set to 1.

MS2 spectrum is represented as a data table containing m/z and corresponding relative intensity. Data tables for two spectra (one from experiment and one from database) are merged by m/z, which yields two equal-length vectors to represent relative intensity for experimental measured MS2 spectrum of u (W_u) and database MS2 spectrum of a_i (W_{a_i}). Cosine similarity (DP) and score for MS2 match ($\mathrm{S}_{\mathrm{MS2}}(u, a_i)$) are defined as below.

$$DP = \frac{\sum W_u W_{a_i}}{\sqrt{\sum W_u^2 \times \sum W_{a_i}^2}} \quad (3)$$

$$\mathrm{S}_{\mathrm{MS2}}(u, a_i) = \mathrm{DP}, \text{ if } \mathrm{DP} > 0.5$$

$$\text{Otherwise } \mathrm{S}_{\mathrm{MS2}}(u, a_i) = 0 \quad (4)$$

(d) $\mathrm{S}_{\mathrm{database}}(u, a_i)$ is positive if the annotated formula a_i exists in HMDB. We give a positive score to a primary seed node annotation if that annotated formula exists in HMDB.

$$\mathrm{S}_{\mathrm{database}}(u, a_i) = 0.5, \text{ if } a_i \text{ in HMDB}$$

$$\text{Otherwise, } \mathrm{S}_{\mathrm{database}}(u, a_i) = 0 \quad (5)$$

(e) $\mathrm{S}_{\mathrm{missing\_isotope}}(u, a_i)$ is negative if an isotopic peak is missing. We penalize a formula annotation if it

passes the intensity threshold (default at $5x10^4$) but does not have isotopic peaks of specified elements. The default isotope being evaluated is ^{37}Cl. Any other elements, such as ^{13}C or ^{18}O, can be included by users.

$$S_{\text{missing\_isotope}}(u, a_i) = -1, \text{ if isotopic peak is missing}$$
$$\text{Otherwise } S_{\text{missing\_isotope}}(u, a_i) = 0 \quad (6)$$

(f) $S_{\text{rule}}(u, a_i)$ is negative if annotation a_i violates basic chemical rules. We strongly penalize formulae that violate basic chemical rules, including a negative RDBE (ring and double bond equivalents), and unlikely element ratios in metabolites (O/P < 3, O/Si < 2).

$$S_{\text{rule}}(u, a_i) = -10, \text{ if chemical rules are violated}$$
$$\text{Otherwise, } S_{\text{rule}}(u, a_i) = 0 \quad (7)$$

(g) $S_{\text{derivative}}(u, a_i)$ is a non-negative score that reflects annotation a_i for node u gains confidence that derived from its parent node *p* with candidate annotation *h*. This is particularly helpful in annotating abiotic peaks. For example, annotation of glutamate sodium adduct will be given a positive $S_{\text{derivative}}$ when its parent node is annotated as glutamate with high score $S_{\text{parent}}(p, h)$. $S_{\text{parent}}(p, h)$ is calculated by summing up scores in (a)-(f).

$$S_{\text{derivative}}(u, a_i) = S_{\text{parent}}(p, h) - 0.5, \text{ if } S_{\text{parent}}(p, h) > 0.5$$
$$\text{Otherwise, } S_{\text{derivative}}(u, a_i) = 0 \quad (8)$$
$$S_{\text{parent}}(p, h) = S_{\text{m/z}}(p, h) + S_{\text{RT}}(p, h) + S_{\text{MS2}}(p, h) + S_{\text{database}}(p, h) + S_{\text{missing\_isotope}}(p, h) + S_{\text{rule}}(p, h) \quad (9)$$

A final score S(u, a_i) for each candidate annotation a_i of node *u* is calculated by summing scores in (a)-(g).

$$S(u, a_i) = S_{\text{m/z}}(u, a_i) + S_{\text{RT}}(u, a_i) + S_{\text{MS2}}(u, a_i) + S_{\text{database}}(u, a_i) + S_{\text{missing\_isotope}}(u, a_i) + S_{\text{rule}}(u, a_i) + S_{\text{derivative}}(u, a_i) \quad (10)$$

Note that for each node *u*, no annotation is always a candidate option. The node score for this null annotation is 0 at default. It can also be set to a positive value to decrease false positive annotations or a negative value to promote increased annotation completeness (at the expense of more false positives).

Scoring candidate edge annotations

The edge scoring system aims to assign high scores to edge annotations that correctly capture biochemical connections between metabolites (based on MS2 spectra similarity) and abiotic connections between metabolites and their mass spectrometry phenomena derivatives, such as isotopes and adducts. Biochemical, isotope, and adduct edge annotations are the most common types. Other less common abiotic connection types are described in a subsequent section.

Suppose we consider two nodes *u* and *v* that are connected by an edge (*u*, *v*). For each pair of nodes *u* and *v* such that there is an edge (*u*, *v*), let the set of candidate formula for node *u* and *v* be denoted as $\{a_1 \dots a_i \dots a_m\}$ and $\{b_1 \dots b_j \dots b_n\}$, respectively, and let the set of candidate atom differences for edge (*u*, *v*) be $\{D_1 \dots D_k \dots D_l\}$. Let $S(u, v, a_i, b_j, D_k)$ be the score of choosing candidate formula a_i for node *u*, candidate formula b_j for node *v* and candidate atom difference D_k for edge (*u*, *v*). Note that $S(u, v, a_i, b_j, D_k)$ is set to be 0 if atom difference D_k does not represent the formula difference of a_i and

b_j.

$$\mathrm{S}(u, v, a_i, b_j, D_k) = 0, \text{ if } |a_i - b_j| \neq D_k \quad (11)$$

Different scoring components for candidate edge annotations are defined as below:

(h) When node *u* and *v* have experimental measured MS2 spectra, $\mathrm{S}_{\mathrm{MS2\_similarity}}(u, v, a_i, b_j, D_k)$ is defined for a biochemical edge, and is a positive score if two connected nodes *u* and *v* have MS2 similarity. $\mathrm{S}_{\mathrm{MS2\_similarity}}$ is determined using the cosine similarity of the MS2 spectra (DP), as described in previous section, and the neutral ion loss similarity (DP_R) in the MS2 spectra[1]. In calculating DP_R, data tables for two spectra (one from node u and one from node v) are merged by [precursor m/z – fragment m/z]. Two equal-length vectors representing the relative intensity of measured MS2 spectrum of u and v are denoted as R_u and R_v respectively. $\mathrm{S}_{\mathrm{MS2\_similarity}}$ is set at 0 for abiotic edges.

$$\mathrm{DP} = \frac{\sum W_u W_v}{\sqrt{\sum W_u^2 \times \sum W_v^2}} \quad (12)$$

$$\mathrm{DP\_R} = \frac{\sum R_u R_v}{\sqrt{\sum R_u^2 \times \sum R_v^2}} \quad (13)$$

$$\mathrm{S}_{\mathrm{MS2\_similarity}}(u, v, a_i, b_j, D_k) = \max(\mathrm{DP}, \mathrm{DP\_R}), \text{ if } \max(\mathrm{DP}, \mathrm{DP\_R}) > 0.3$$
$$\text{Otherwise, } \mathrm{S}_{\mathrm{MS2\_similarity}}(u, v, a_i, b_j, D_k) = 0 \quad (14)$$

(i) $\mathrm{S}_{\mathrm{co\_elution}}(u, v, a_i, b_j, D_k)$ is defined for an abiotic edge, and is a negative score if the RT of two connected nodes differ more than a threshold (0.05 min), given the formula difference of a_i and b_j matches the atom difference defined by D_k. $\mathrm{S}_{\mathrm{co\_elution}}$ is set at 0 for biochemical edges.

$$\mathrm{S}_{\mathrm{co\_elution}}(u, v, a_i, b_j, D_k) = -5 \times |u_{\mathrm{RT}} - v_{\mathrm{RT}}|, \text{ if } |u_{\mathrm{RT}} - v_{\mathrm{RT}}| \geq 0.05 \text{ min}$$
$$\text{Otherwise, } \mathrm{S}_{\mathrm{co\_elution}}(u, v, a_i, b_j, D_k) = 0 \quad (15)$$

(j) $\mathrm{S}_{\mathrm{type}}(u, v, a_i, b_j, D_k)$ is defined for all edges, given the formula difference of a_i and b_j matches the atom difference defined by D_k, and is a non-negative score depending on the connection type of edge, which is defined by D_k, including biotransformation, adduct, isotope and fragment (Supplementary Table 1, 2). The magnitude of scores reflects the empirical confidence in the annotation type when certain atom differences occur, and can be adjusted based on user preferences.

$$\mathrm{S}_{\mathrm{type}}(u, v, a_i, b_j, D_k) = 0, \text{ if } D_k \in \text{biotransformation}$$
$$\mathrm{S}_{\mathrm{type}}(u, v, a_i, b_j, D_k) = 0.5, \text{ if } D_k \in \text{adduct}$$
$$\mathrm{S}_{\mathrm{type}}(u, v, a_i, b_j, D_k) = 2, \text{ if } D_k \in \text{isotope}$$
$$\mathrm{S}_{\mathrm{type}}(u, v, a_i, b_j, D_k) = 0.3, \text{ if } D_k \in \text{common neutral loss} \quad (16)$$

(k) For each D_k∈ isotope, $S_{isotope\_intensity}(u, v, a_i, b_j, D_k)$ is defined for isotope edge (*u, v*) where b_j is the isotopic derivative of a_i with atom difference of D_k, and is a negative score if the measured isotope peaks deviate from expected natural abundance. The score for an isotope edge depends on how likely the ratio of measured and expected isotopic intensity ($Ratio_{isotope}$) is observed in an empirical normal distribution $N(1, \sigma^2_{isotope})$. Isotopes of all elements included in the atom difference table are evaluated.

$$\text{Ratio}_{\text{isotope}} = \frac{v_{\text{intensity}} \,/\, u_{\text{intensity}}}{\text{Expected isotopic intensity ratio } (a_i, b_j, D_k)} \tag{17}$$

$$S_{isotope\_intensity}(u, v, a_i, b_j, D_k) = log_{10}\left[\frac{normPDF(\text{Ratio}_{\text{isotope}}, 1, \sigma^2_{\text{isotope}})}{normPDF(1, 1, \sigma^2_{\text{isotope}})}\right] \tag{18}$$

normPDF(x, μ, $\sigma^2_{isotope}$) is defined as the normal probability density function with mean μ and variance $\sigma^2_{isotope}$ evaluated at the values in x. $\sigma_{isotope}$ is empirically defined as below, so that when measured isotope intensity is close to detection limit, a larger $\sigma_{isotope}$ (a widened distribution, which is more tolerant to discrepancy) will be used.

$$\sigma_{\text{isotope}} = 0.2 + 10^{3 - log_{10}(v_{\text{intensity}})} \tag{19}$$

A final edge annotation score $S(u, v, a_i, b_j, D_k)$ for choosing candidate formula a_i for node *u*, candidate formula b_j for node *v* and candidate atom difference D_k for edge (*u*, *v*) is calculated by summing scores in (h)-(n).

$$\begin{aligned} S(u, v, a_i, b_j, D_k) = {} & S_{\text{MS2\_similarity}}(u, v, a_i, b_j, D_k) + S_{\text{co\_elution}}(u, v, a_i, b_j, D_k) + \\ & S_{\text{type}}(u, v, a_i, b_j, D_k) + S_{\text{isotope\_intensity}}(u, v, a_i, b_j, D_k) \end{aligned} \tag{20}$$

Less common edge annotations

LC-MS metabolomics may include additional abiotic relationships. In orbitrap data, these include oligomers, multi-charge species, heterodimers, in-source fragments of known or unknown metabolites[3], and ringing artifact peaks surrounding high intensity ions[4,5]. These relationships were included in NetID as additional edge types, which are evaluated for all m/z pairs within a predefined RT range (0.2 min). Associated scores are provided at the end of the section.

(l) Oligomer and multi-charge species. An oligomer/multi-charge edge is assigned between two nodes *u* and *v*, if their m/z satisfy

$$|v_{\text{m/z}} - \text{n} \times u_{\text{m/z}}| < u_{\text{m/z}} \times 10 \text{ ppm}, \text{ n} \in \{\text{positive integers}\} \tag{21}$$

(m) Heterodimer. Heterodimer peak (node *v*) may be observed when one abundant metabolite (node *u*) forms ion cluster with other ion species (node *t*). We examine nodes that have intensity above 10^5, and assign a heterodimer edge between two nodes *u* and *v* if their m/z difference satisfy

$$|(v_{\text{m/z}} - u_{\text{m/z}}) - t_{\text{m/z}}| < u_{\text{m/z}} \times 10 \text{ ppm} \tag{22}$$

(n) In-source fragments. Such peaks may be observed when one abundant metabolite breaks up into fragments during the ionization process.

Database MS2 of known metabolites can be used to identify known ion fragment peaks[3]. If candidate annotation b_j of node *v* is annotated with a HMDB ID associated with database MS2 spectrum, and m/z

of node *u* matches to a fragment m/z in b_j's MS2 spectrum, then a database fragment edge will connect such two nodes. That is,

$$u_{\mathrm{m/z}} \in \text{Database MS2 spectrum of candidate annotation } b_j \text{ of node } v \quad (23)$$

Measured MS2 spectra can also be used to identify fragment peaks (including covering unknowns not present in MS2 database). If node *v* is associated with a measured MS2 spectrum, and m/z of another node *u* matches to a fragment m/z in the MS2 spectra, then an experiment fragment edge will connect such two nodes. That is,

$$u_{\mathrm{m/z}} \in \text{Measured MS2 spectrum of node } v \quad (24)$$

(o) Ringing artifacts. Ringing peaks are artifact peaks (node *v*) often observed on both sides of the m/z of an intense ion peak (node *u*) in Fourier-transformed MS instrument including orbitrap. We examine nodes that have intensity above 10^6, and assign a ringing artifact edge between two nodes if two nodes satisfy

$$50 \text{ ppm} < | v_{\mathrm{m/z}} - u_{\mathrm{m/z}} | / u_{\mathrm{m/z}} < 1000 \text{ ppm}$$
$$u_{\mathrm{intensity}} / v_{\mathrm{intensity}} > 50 \quad (25)$$

Scoring of these additional abiotic edges follow the same rules described in the "Scoring edge annotations" section with additional S_{type} defined as below.

$$\mathrm{S_{type}}(u, v, a_i, b_j, D_k) = 0.5, \text{ if } D_k \in \text{oligomer or multi-charge}$$
$$\mathrm{S_{type}}(u, v, a_i, b_j, D_k) = 0, \text{ if } D_k \in \text{heterodimer}$$
$$\mathrm{S_{type}}(u, v, a_i, b_j, D_k) = 0.3, \text{ if } D_k \in \text{database MS2 fragment}$$
$$\mathrm{S_{type}}(u, v, a_i, b_j, D_k) = 1, \text{ if } D_k \in \text{measured MS2 fragment}$$
$$\mathrm{S_{type}}(u, v, a_i, b_j, D_k) = 2, \text{ if } D_k \in \text{ringing artifacts} \quad (26)$$

A final edge annotation score $S(u, v, a_i, b_j, D_k)$ for choosing candidate formula a_i for node *u*, candidate formula b_j for node *v* and candidate atom difference D_k for edge (*u*, *v*) is calculated by summing scores in (h)-(o).

$$\mathrm{S}(u, v, a_i, b_j, D_k) = \mathrm{S_{MS2\_similarity}}(u, v, a_i, b_j, D_k) + \mathrm{S_{co\_elution}}(u, v, a_i, b_j, D_k) + \mathrm{S_{type}}(u, v, a_i, b_j, D_k) + \mathrm{S_{isotope\_intensity}}(u, v, a_i, b_j, D_k) \quad (27)$$

Parameters to edit in different usage situations

Currently the algorithm is developed using Thermo Orbitrap instruments results. We anticipate the algorithm will work for other high mass accuracy data, such as TOF data. Due to the difference in mass resolving power and mass accuracy between TOF and orbitrap, in applying NetID to TOF data, it would be important to check parameters related to mass accuracy, e.g. the m/z tolerance for seed matching and candidate node annotations, and the score evaluating m/z accuracy for candidate node annotations. Other data acquisition differences may also impact the optimal parameter settings in NetID. For example, if sample type (e.g. yeast vs mouse) is different, users may check on choice of reference library and if ionization settings (e.g. high vs low in-source CID) is different, users may check on the scoring parameters related to forming clustered ion species such as oligomers or heterodimers. The overall logics is to assign high scores to those candidate annotations that effectively align the experimentally observed ion peaks with prior metabolomics knowledge, so the network optimization will lead to an optimal annotation.

Supplementary Note 2 – Glucosyl-taurine synthesis

Glucosyl-taurine synthesis was carried out following previous literature reports with slight modifications[6]. In brief, dry methanol was obtained by distillation of HPLC-grade methanol (Fisher; HPLC grade 0.2 micron filtered) over CaH_2 (Acros Organics; ca. 93% extra pure, 0-2 mm grain size). A flame-dried round-bottom flask equipped with a reflux condenser and stir bar was charged with 2.0 g taurine (Alfa Aesar; 99%), 3.1 g D-glucose (Acros Organics; ACS reagent), and 80 mL of dry methanol. This mixture was sonicated under an inert atmosphere for 30 min before being returned to the manifold for the reaction. To the fine-suspension of taurine and glucose in dry methanol at room temperature, 4.0 mL 5.4 M sodium methoxide in methanol (Acros Organics) was added via glass syringe. At this point, the suspension began to dissolve and after 30 minutes, gave a clear and colorless solution. The solution was stirred vigorously under an inert atmosphere for 72 hours, which resulted in a faint peach-colored solution. This solution was chilled to 0 ℃, and ~200 mL of absolute ethanol (200 proof) was added and precipitation was allowed to occur at this temperature for 30 minutes. Solvent was then removed by filtration over a glass filter (medium porosity), and washed with ~100 mL of absolute ethanol, affording a fine pale-yellow powder (2.4 g; crude material).

NMR was carried out to validate the structure of synthesized N-glucosyl-taurine. Selective TOCSY experiments using DIPSI2 spin-lock and with added chemical shift filter[7] were run on a Bruker Avance III HD NMR spectrometer equipped with a custom-made QCI-F cryoprobe (Bruker, Billerica, MA) at 800 MHz and at 295 K controlled temperature. The sample was dissolved in DMSO-d6. The spectra shown on the plots are results of 200 ms SL mixing, 8 scans each. Data processing (MNova v.14, Mestrelab Research S.L., Santiago de Compostela, Spain) included zero filling, 1 Hz Gaussian apodization, phase- and baseline correction. NMR analysis suggests that the final crude material contains 5.2% N-glucosyl-taurine mixed with unreacted substrates (Extended Data Fig. 8).

Supplementary references:

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Supplementary Note 3 - NetID User Guide

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8 August 2021

Liquid chromatography-high resolution mass spectrometry (LC-MS)-based metabolomics aims to identify and quantitate all metabolites, but most LC-MS peaks remain unidentified. Here, we present a global network optimization approach, NetID, to annotate untargeted LC-MS metabolomics data. The approach aims to generate, for all experimentally observed ion peaks, annotations that match the measured masses, retention times, and (when available) MS/MS fragmentation patterns. Peaks are connected based on mass differences reflecting adducting, fragmentation, isotopes, or feasible biochemical transformations. Global optimization generates a single network linking most observed ion peaks, enhances peak assignment accuracy, and produces chemically-informative peak-peak relationships, including for peaks lacking MS/MS spectra. Applying this approach to yeast and mouse data, we identified five previously unrecognized metabolites (thiamine derivatives and N-glucosyl-taurine). Isotope tracer studies indicate active flux through these metabolites. Thus, NetID applies existing metabolomic knowledge and global optimization to substantially improve annotation coverage and accuracy in untargeted metabolomics datasets, facilitating metabolite discovery.

NetID requires (1) a peak table (in .csv format) containing m/z, RT and intensity from high-resolution mass spectrometry data; (2) a reference compound database, for which we provide HMDB, YMDB, a lite version of PubChem (PubChemLite.0.2.0) and a subset of 47,101 biopathway related entries (PubChemLite_Bio) that the user may choose; and. (3) a transformation table (in .csv format), for which we assembled a list of 25 biochemical atom differences and 59 abiotic atom differences. NetID optionally use (4) a list of excel files containing MS2 fragmentation information (m/z and intensity) for peaks in the above peak table and (5) a list of known metabolites' retention time, for which we provide our in-house retention time list for demonstration. Users can customize the compound database, the transformation table and the retention time list following the user guide. Currently the algorithm is developed using Thermo Orbitrap instruments results. We anticipate the algorithm will work for other high mass accuracy data, such as TOF data, but parameters may need to be optimized for the best performance.

Citation: https://www.biorxiv.org/content/10.1101/2021.01.06.425569
Git-hub: https://github.com/LiChenPU/NetID

1 Environment Setup

This section provides step-by-step instructions to set up the environment to run NetID algorithm in a local computer. A Windows system is recommended. Typical install time on a "normal" desktop computer is within a few hours.

1.1 Software installation

- Install R, Rstudio, Rtools40, ILOG CPLEX Optimization Studio (CPLEX) and Git, preferably at default location.

R(version 4.0, 4.1 tested): https://www.r-project.org/
RStudio: https://rstudio.com/products/rstudio/download
Rtools40: https://cran.r-project.org/bin/windows/Rtools/ow
CPLEX(version 12.8,12.10,20.10 tested): https://www.ibm.com/academic/technology/data-science
Git: https://git-scm.com/downloads

1.2 Code download

1.2.1 Via Git (recommended)

1. Install **git** via https://support.rstudio.com/hc/en-us/articles/200532077?version=1.3.1093&mode=desktop
2. In Rstudio, go to `File` → `New project` → `Version control` → `Git`, enter https://github.com/LiChenPU/NetID.git for `URL`, select a subdirectory, and create project.
3. You should be able to see all files in place under your selected subdirectory. Use pull option to check for latest updates.

1.2.2 Via Github

1. Go to website https://github.com/LiChenPU/NetID, hit the green `code` button, select download zip, and unzip files.

1.3 Package dependency installation

1.3.1 Install common packages

1. Open the R script `NetID_packages.R` in `get started` folder.
2. Run all lines.
3. Run all lines again. If you see "No new packages added...", then it means all packages are successfully installed.

1.3.2 Install cplexAPI

The package, **cplexAPI**, connecting R to CPLEX, requires additional installation steps.

1. Go to website: https://cran.r-project.org/web/packages/cplexAPI/index.html, look for `Package source`, and download `cplexAPI_1.4.0.tar.gz`. In the same page, look for `Materials`, a package installation guide can be found in the link `INSTALL`.
2. Unzip the folder `cplexAPI` to the **desktop**, open subfolder `src`, follow the installation guide to modify the file `Makevars.win`.
   Note: Replace `\` in the `Makevars.win` file into `/` in order for R to recognize the path.
   - For example, the `-I"${CPLEX_STUDIO_DIR}\cplex\include"` needs to be replaced with the path CPLEX_studio is installed, such as:
     `-I"C:/Program Files/IBM/ILOG/CPLEX_Studio1210/cplex/include"`
   - The `-L"${CPLEX_STUDIO_LIB}"` needs to be replaced with the path CPLEX_studio is installed, such as:
     `-L"C:/Program Files/IBM/ILOG/CPLEX_Studio1210/cplex/bin/x64_win64"`

- The last part "-lcplexXXX" needs to be replaced with specific version code. For example, use "-lcplex12100" for CPLEX_Studio1210, and "-lcplex2010" for CPLEX_Studio201

3. build package,
   - In command line, change `${Username}` to the actual user name and run line below, `R CMD build --no-build-vignettes --no-manual --md5 "C:\Users\${Username}\Desktop\cplexAPI"`
   - Alternatively, you can run the lines below in Rstudio: `setwd('C:/Users/${Username}/Desktop/cplexAPI')` `devtools::build(vignettes = FALSE)`

   a new package `cplexAPI_1.4.0.tar.gz` will be built under the default path (for example, `C:\Users\${Username}`)

   Note: You need to add R and Rtools40 to Environmental Variables PATH, with instruction provided at the end.

4. In command line, run line below to install package.
   `R CMD INSTALL --build --no-multiarch .\cplexAPI_1.4.0.tar.gz`
   If you see `DONE (cplexAPI)`, then the package installation is successful.
   Note: if error occurs relating to `__declspec(dllimport deprecated)`, you need to go to `C:\Program Files\IBM\ILOG\CPLEX_Studio1210\cplex\include\ilcplex` (or your own installation folder), open the file `cpxconst.h`, go to the line indicated in the error message or search for `__declspec(dllimport deprecated)`, add `_` in between, make it to `__declspec(dllimport_deprecated)`. Save file and repeat *step 4*.

5. To test if cplexAPI is installed properly and to take a short venture using CPLEX in R, refer to **Package cplexAPI – Quick Start** in https://cran.r-project.org/web/packages/cplexAPI/index.html.

2 Using NetID

This section will use yeast negative-mode dataset and mouse liver negative-mode dataset as examples to walk through the NetID workflow.

Note 1: If other El-MAVEN version was used, check the "raw_data.csv" for the column number where the first sample is located, and specify that in the NetID_run_script.R file. For example, In El-MAVEN (version 7.0), first_sample_col_num is set at 15 as default. If El-MAVEN (version 12.0) is used, first_sample_col_num should be set at 16.

Note 2: for more advanced uses, scoring and other parameters can be edited in NetID_function.R and NetID_run_script.R. Read the manuscript method section for detailed explanation on parameters.*

2.1 Yeast negative-mode dataset

In the `Sc_neg` folder, file `raw_data.csv` is the output from **Elmaven** recording MS information, and is the input file for **NetID**. MS2 is not collected for this dataset.

2.1.1 Running the code

1. Open `code folder` → `NetID_run_script.R`

2. In the `# Setting path #### section`, set work_dir as `"../Sc_neg/"`.

```
# Setting path ####
{
  setwd(dirname(rstudioapi::getSourceEditorContext()$path))
  source("NetID_function.R")

  work_dir = "../Sc_neg/"
  setwd(work_dir)
  printtime = Sys.time()

}
```

3. In the `# Read data and files #### section`, set filename as `"raw_data.csv"`, set MS2_folder as `""`.
   set `ion_mode` as `-1` if negative ionization data is loaded, and `1` if positive ionization data loaded.

```
# Read data and files ####
{
  Mset = list()
  # Read in files
  Mset = read_files(filename = "raw_data.csv",
                    LC_method = "Hilic_25min_QE",
                    ion_mode = -1 # 1 for pos mode and -1 for neg mode
                    )
  Mset = read_MS2data(Mset,
                      MS2_folder = "") # MS2
}
```

4. Keep all other parameters as default, and run all lines.

2.1.2 Expected outputs

1. In the console, error message should not occur. If optimization step is successful, you will see messages in the following format.

```
"Optimization ended successfull - integer optimal, tolerance - OBJ_value = 2963.71
(bestobjective - bestinteger) / (1e-10 + |bestinteger|) = 0.000048268"
95.74 sec elapsed
```

2. Three files will be generated in the `Sc_neg` folder. Expected run time on a "normal" desktop computer should be within an hour.
   - `NetID_output.csv` contains the annotation information for each peak.
   - `NetID_output.RData` contains node, edge and network information. The file will be used for network visualization in Shiny R app.
   - `.RData` records the environmental information after running codes. The file is mainly used for development and debugging.

2.2 Your own dataset

2.2.1 MS1 dataset preparation

1. File conversion. Use software **ProteoWizard40** (version 3.0.11392) to convert LC-MS raw data files (.raw) into mzXML format. A command line script specifies the conversion parameter. Assuming the raw data are in `D:/MS data/test`. Type in the scripts below.

```
D:
cd D:/MS data/test
"C:/Program Files/ProteoWizard/ProteoWizard 3.0.11392/msconvert.exe"
*.raw --filter "peakPicking true 1-" --simAsSpectra --srmAsSpectra --mzXML
```

If **ProteoWizard** is installed in location other than `C:/Program Files/ProteoWizard/ProteoWizard 3.0.11392/msconvert.exe`, specify your path to where you can find the `msconvert.exe` file. Expected outputs will be `.mzXML` files from `.raw` data.

2. **El-MAVEN (version 7.0)** is used to generate a peak table containing m/z, retention time, intensity for peaks. Detailed guides for peak picking can be found in https://elucidatainc.github.io/ElMaven/faq/.
   After peak picking and a peak table tab has shown up, click `export to CSV`. Choose `export all groups`. In the pop-up saved window, choose format `Groups Summary Matrix Format Comma Delimited`. Save to the desired path.

3. Under the `NetID` folder, create a new folder `NetID_test`, copy the csv file from *step 2* into the folder, and change the filename into `raw_data.csv`.

2.2.2 MS2 dataset preparation

NetID currently utilizes targeted MS2 data for better MS2 quality, and will incorporate data-dependent MS2 data in the future.

1. Prepare MS2 inclusion list
   For targeted MS2 analysis, from the peak list generated in *step 1*, select the peaks (m/z, RT) that you want to perform MS2, and arrange them into multiple csv files that will serve as the inclusion lists to set up the PRM method on **Thermo QExactive** instrument. Instruction can be found in https://proteomicsresource.washington.edu/docs/protocols05/PRM_QExactive.pdf.
   Note: Arrange the parent ions so as to avoid to perform many PRMs at same time. An example is shown below with the start and End time set as RT-1.5 and RT+1.5 (min) to have good chromatogram coverage.

```
library(readr)
read_csv("example.csv")
##
## -- Column specification --------------------------------------------------------
## cols(
##   Mass = col_double(),
##   Formula = col_logical(),
##   Formula_type = col_logical(),
##   Species = col_logical(),
##   CS = col_logical(),
##   Polarity = col_character(),
##   Start = col_double(),
##   End = col_double(),
##   CE = col_double(),
```

```
##   CE_type = col_character(),
##   MSXID = col_logical(),
##   Comment = col_character()
## )
## # A tibble: 16 x 12
##     Mass Formula Formula_type Species CS    Polarity  Start   End    CE CE_type
##    <dbl> <lgl>   <lgl>        <lgl>   <lgl> <chr>     <dbl> <dbl> <dbl> <chr>
##  1 499.  NA      NA           NA      NA    Negative  0.456  3.46    30 NCE
##  2 722.  NA      NA           NA      NA    Negative  0.733  3.73    30 NCE
##  3 403.  NA      NA           NA      NA    Negative  1.06   4.06    30 NCE
##  4 211.  NA      NA           NA      NA    Negative  1.20   4.20    30 NCE
##  5 328.  NA      NA           NA      NA    Negative  1.40   4.40    30 NCE
##  6 149.  NA      NA           NA      NA    Negative  1.59   4.59    30 NCE
##  7 151.  NA      NA           NA      NA    Negative  2.69   5.69    30 NCE
##  8 335.  NA      NA           NA      NA    Negative  2.70   5.70    30 NCE
##  9 143.  NA      NA           NA      NA    Negative  4.07   7.07    30 NCE
## 10  89.0 NA      NA           NA      NA    Negative  5.67   8.67    30 NCE
## 11 283.  NA      NA           NA      NA    Negative  6.92   9.92    30 NCE
## 12 202.  NA      NA           NA      NA    Negative  8.79  11.8     30 NCE
## 13 160.  NA      NA           NA      NA    Negative 10.3   13.3     30 NCE
## 14 216.  NA      NA           NA      NA    Negative 11.4   14.4     30 NCE
## 15 125.  NA      NA           NA      NA    Negative 12.0   15.0     30 NCE
## 16 230.  NA      NA           NA      NA    Negative 12.9   15.9     30 NCE
## # ... with 2 more variables: MSXID <lgl>, Comment <chr>
```

2. Instrument setup
   Set up the **QExactive** instrument so that it contains both "Full MS" and "PRM" scan events. For PRM setup, use the above file as inclusion list to perform targeted MS2 analysis. We typically use the following setting for MS2 analysis: resolution 17500, AGC target 1e6, Maximum IT 500 ms, isolation window 1.5 m/z. For a total of 1500 parent ions and 15 parent ions for each method, it requires a total of 100 runs, or ~42 hours using a 25-min LC method.

3. MS2 file conversion.
   RawConverter (version 1.2.0.1, http://fields.scripps.edu/rawconv/) is used to convert the `.raw` file into `.mzXML` file that contains MS2 information. Keep the default parameters except setting `Environment Type` as `Data Independent`, and `Output Formats` as `mzXML`.

4. MS2 reading and cleaning.
   A matlab code is used for MS2 reading and cleaning, which can be found in **CodeOcean** as a published capsule (https://codeocean.com/capsule/1048398/tree/v1). The `csv` files from *1* paired with the MS2 data files in `mzXML` format from *3* are the required input data. Refer to capsule description and `readme.md` file for more details of how the code works. In Brief,

   - Prepare filename. Filenames for both csv and mzXML files should be named as `prefixNNN`, where prefix is the given file name and NNN is the 3 digits number in continuous order (e.g. `M001.csv`, `M002.csv`,... and `M001.mzXML`, `M002.mzXML`,... in the `/data` folder).

   - Duplicate the capsule to your own account so you can edit and use the capsule. Upload your own files and remove the previous files in `/data` folder.

- Specify the prefix and the range of numbers at the beginning section of the main code `Main_example.m`.
- Set the main code as file to run in Code Ocean using the dropdown menu next to main code.
- Click `reproducible run` to perform the batch processing.
- The resulting output files in `.xlsx` format with the same filenames will appear in the timeline. Each `xlsx` file contains multiple tabs of cleaned MS2 spectra. The names of the tabs correspond to the row numbers of the `csv` file specifying the individual parent peak information.

5. Save files to folders.
   Back to the `NetID_test` folder, create a new folder `MS2`, download all `xlsx` files from *4* into the folder.

2.2.3 Running the code

1. Open `code` folder → `NetID_run_script.R`.
2. In the `# Setting path #### section`, set `work_dir` as `"../NetID_test/"`.
3. In the `# Read data and files ####` section,
   set `filename` as `raw_data.csv`, set `MS2_folder` as `MS2`.
   set `LC_method` to specify column to read for the retention time of known standards. (In folder `NetID` → `dependent` → `known_library.csv`, update the retention time info as needed.)
   set `ion_mode` as `-1` if negative ionization data is loaded, and `1` if positive ionization data loaded.
4. Keep all other parameters as default, and run all lines.

2.2.4 Expected outputs

Similar to the `demo` file, the console will print out message indicating optimization step is successful, and three files `NetID_output.csv`, `NetID_output.RData` and `.RData` will be generated in the `NetID_test` folder

2.3 Other Settings

2.3.1 Compound librarys

2.3.1.1 Other provided librarys

NetID provides 4 librarys for the user to choose: **HMDB**, **YMDB**, **PubChem**, **PubChem Bio-pathway only**.

To select the desired database, change `HMDB_library_file = "../dependent/hmdb_library.csv"` to `../dependent/ymdb_library.csv`, `../dependent/pbcm_library.csv` or `../dependent/pbcm_library_bio.csv`.

```
Mset = read_files(filename = "raw_data.csv",
                  LC_method = "Hilic_25min_QE",
                  ion_mode = -1, # 1 for pos mode and -1 for neg mode
                  HMDB_library_file = "../dependent/hmdb_library.csv"
```

```
                          )
```

2.3.1.2 Design your own library

1. A workable library requires following columns.

```
read_csv("../../dependent/hmdb_library.csv")
##
## -- Column specification --------------------------------------------------------
## cols(
##   accession = col_character(),
##   iupac_name = col_character(),
##   name = col_character(),
##   SMILES = col_character(),
##   status = col_character(),
##   formula = col_character(),
##   mass = col_double(),
##   rdbe = col_double(),
##   category = col_character()
## )
## # A tibble: 114,014 x 9
##    accession  iupac_name     name  SMILES    status formula  mass  rdbe category
##    <chr>      <chr>          <chr> <chr>     <chr>  <chr>   <dbl> <dbl> <chr>
##  1 HMDB00000~ (2S)-2-amino-~ 1-Me~ CN1C=NC(~ quant~ C7H11N~ 169.      4 Metabol~
##  2 HMDB00000~ propane-1,3-d~ 1,3-~ NCCCN     quant~ C3H10N2  74.1     0 Metabol~
##  3 HMDB00000~ 2-oxobutanoic~ 2-Ke~ CCC(=O)C~ quant~ C4H6O3  102.      2 Metabol~
##  4 HMDB00000~ 2-hydroxybuta~ 2-Hy~ CCC(O)C(~ quant~ C4H8O3  104.      1 Metabol~
##  5 HMDB00000~ (1S,10R,11S,1~ 2-Me~ [H][C@@]~ quant~ C19H24~ 300.      8 Metabol~
##  6 HMDB00000~ (3R)-3-hydrox~ (R)-~ C[C@@H](~ quant~ C4H8O3  104.      1 Metabol~
##  7 HMDB00000~ 1-[(2R,4S,5R)~ Deox~ OC[C@H]1~ quant~ C9H12N~ 228.      5 Metabol~
##  8 HMDB00000~ 4-amino-1-[(2~ Deox~ NC1=NC(=~ quant~ C9H13N~ 227.      5 Metabol~
##  9 HMDB00000~ (1S,2R,10R,11~ Cort~ [H][C@@]~ quant~ C21H30~ 346.      7 Metabol~
## 10 HMDB00000~ (1S,2R,10S,11~ Deox~ [H][C@@]~ quant~ C21H30~ 330.      7 Metabol~
## # ... with 114,004 more rows
```

2. To build your own library, make a csv file in the same format as the one shown above, and set `HMDB_library_file = "../dependent/hmdb_library.csv"` to your desired directory in `NetID_run_script.R`.

2.3.2 Modifying `emperical_rules.csv`

`emperical_rules.csv` can also be created or modifed to support specific biotransformation. A workable emperical_rules requires following columns. * `name` and `note` is not necessary. * `category` includes: `Biotransform`, `Natural_abundance`, `Adduct`, `Fragment` and `Radical` * `rbde` is calculated using the `formula_rbde` function of the package `lc8` * `direction` states the possible direction of transformation: `1` means from larger mass to smaller mass; `0` means the opposite; `-1` means both direction are possible.

```
read_csv("../../dependent/empirical_rules.csv")
##
## -- Column specification --------------------------------------------------------
```

```
## cols(
##   category = col_character(),
##   name = col_character(),
##   formula = col_character(),
##   mass = col_double(),
##   direction = col_double(),
##   rdbe = col_double(),
##   note = col_character()
## )
## # A tibble: 84 x 7
##    category     name  formula    mass direction  rdbe note
##    <chr>        <chr> <chr>     <dbl>     <dbl> <dbl> <chr>
##  1 Biotransform O-HN  O1N-1H-1  0.984         0     0 Deamination
##  2 Biotransform NH3-O N1H3O-1   1.03          0    -1 Transamination
##  3 Biotransform H2    H2        2.02          0    -1 Hydrogenation
##  4 Biotransform CH2   C1H2     14.0           0     0 Methylation
##  5 Biotransform NH    N1H1     15.0           0     0 Amination
##  6 Biotransform O     O1       16.0           0     0 Hydroxylation
##  7 Biotransform N1H3  N1H3     17.0           0    -1 Amination (+NH3)
##  8 Biotransform H2O   H2O1     18.0           0    -1 Hydration
##  9 Biotransform CO    C1O1     28.0           0     1 Formylation (+CO)
## 10 Biotransform C2H4  C2H4     28.0           0     0 Beta oxidation
## # ... with 74 more rows
```

2.3.3 Retention time list

2.3.3.1 Customize your own RT table

1. In the dependent folder, open the `known_library_customized.csv` file

```
read_csv("../../dependent/known_library_customized.csv")[1:5,]
##
## -- Column specification --------------------------------------------------------
## cols(
##   name = col_character(),
##   HMDB = col_character(),
##   formula = col_character(),
##   SMILES = col_character(),
##   Hilic_25min_QE = col_double(),
##   No_RT = col_logical()
## )
## # A tibble: 5 x 6
##   name                      HMDB     formula    SMILES       Hilic_25min_QE No_RT
##   <chr>                     <chr>    <chr>      <chr>                 <dbl> <lgl>
## 1 1-Methyl imidozolacetic~ HMDB000~ C6H8N2O2   CN1C=C(N=C1~           9.05 NA
## 2 5-L-Hydroxytryptophan    <NA>     C11H12N2O3 <NA>                 10.2  NA
## 3 ADP                      <NA>     C10H15N5O~ <NA>                 13.9  NA
## 4 CDP                      <NA>     C9H15N3O1~ <NA>                 NA    NA
## 5 CDP-choline              <NA>     C14H26N4O~ <NA>                 NA    NA
```

2. Column `Name`, `formula` are required. Column `HMDB`, `SMILES` are optional. For each RT list (e.g. Hilic_25min_QE), record the retention time under the column. Multiple RT lists can be stored by adding additional columns. Empty retention time is allowed for a entry.

2.3.3.2 Skip RT table

Setting the LC_method = "No_RT". Then RT information will not be considered in the algorithm.

```
Mset = read_files(filename = "raw_data.csv",
                  LC_method = "No_RT",
                  ion_mode = -1, # 1 for pos mode and -1 for neg mode
                  HMDB_library_file = "../dependent/hmdb_library.csv"
                  )
```

2.3.4 Score Setting

See Supplementary Note 2 of NetID paper for explanation

3 NetID Visualization

This section provides instruction to visualize and explore **NetID** output results in either **Cytoscape** software or interactive **Shiny R app**. After running **NetID** algorithm, it will export one `.R` and two `.csv`files (`cyto_node.csv` and `cyto_edges.csv`), storing the nodes and edges of the output network.

3.1 Cytoscape

0. What is **Cytoscape** For more info regarding what is **Cytoscape**, check https://cytoscape.org/what_is_cytoscape.html.
1. install **Cytoscape** Download **Cytoscape** (https://cytoscape.org/download.html) and follow installation instruction to install onto your computer.
2. Load the example **NetID** output into **Cytoscape**
   - Run **Cytoscape**, click `import network from file system`, and load `cyto_edges.csv`, set `edge_id` column as the `key`, set `node1`as `source node`, set `node2` column as `target node`, and the rest columns as `edge attribute`.
   - Click `import table from file`, load `cyto_node.csv`, set `node_id` column as the `key`, and the rest columns as `node attribute`.
   - Select `subnetwork`, set `styles`, and explore the network with various functionalities inside **Cytoscape**.
3. Explore in Cytoscape
   http://manual.cytoscape.org/en/stable/index.html provides all you need to know about exploring in **Cytoscape**. (This writer knew little about this cool software, so all he could give was this link and *may the Force be with you*.)
4. Export
   The network as well as the curated subnetworks can be exported for future analysis or sharing with others. An example network file `example.cys` is included along with the two `.csv` files, which is created using **Cytoscape** version 3.8.2

3.2 Shiny App

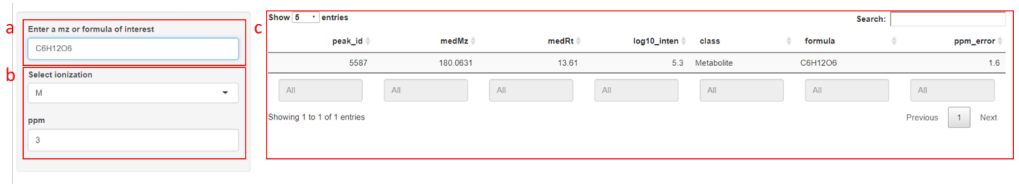
This part provides instruction to visualize and explore **NetID** output results in the interactive **Shiny R app**. A 21-inch or larger screen is recommended for best visualization.

3.2.1 Runing Shiny App

1. Open `code` folder → `R_shiny_App.R`.
2. In the `# Read in files #### section`, set datapath as `../Sc_neg/`
3. Keep all other parameters as default, and run all lines.
4. A Shiny app will pop up.

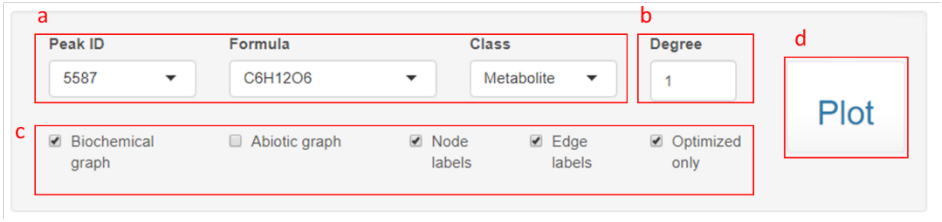
3.2.2 Searching peaks of interest

1. On the left panel, you can enter a m/z or a formula to search your peak of interest. For example, 180.0631 or C6H12O6 will automatically update the data table on the right. Enter 0 to restore full list for the data table.
2. Change ionization and ppm window to adjust calculated m/z. W
3. On the right, you can explore the peak list in an interactive data table, including global text search on top right, specifying ranges for numeric column or searching text within character columns, ranking each column etc.

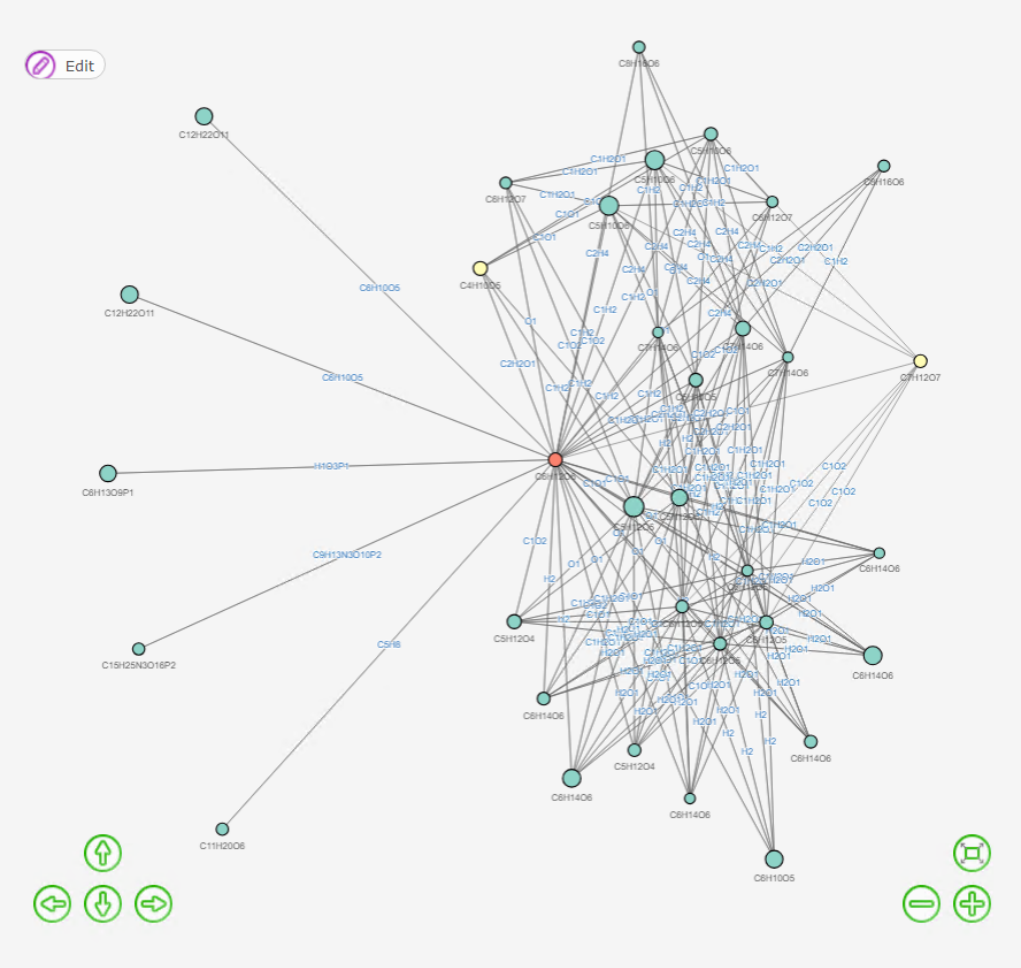


3.2.3 Network Visualization

1. Peak ID, formula and class determines the center node for the network graph. Peak ID will be automatically updated by the first line in the data table if a m/z or formula is given. Alternatively, you can manually enter Peak ID.
2. The degree parameter controls how far the network expands from the center node. Degree 1 means only nodes directly connected to the center node will be shown and degree 2 means nodes connected to degree 1 will be shown, etc.
3. Biochemical graph shows biochemical connections. Abiotic graph shows abiotic connections. Node labels and Edge labels determines if the graph show node or edge labels. Optimized only determines whether to show only the optimal annotations or all possible annotations in the network.
4. When setting parameters, hit plot to see the network graph.



5. A sample network graph is shown below (a different center node may give less complicated graph). You may edit the nodes or edges (top left), move figures with the arrow buttons (bottom left), and zoom in/out or center figure (bottom right).

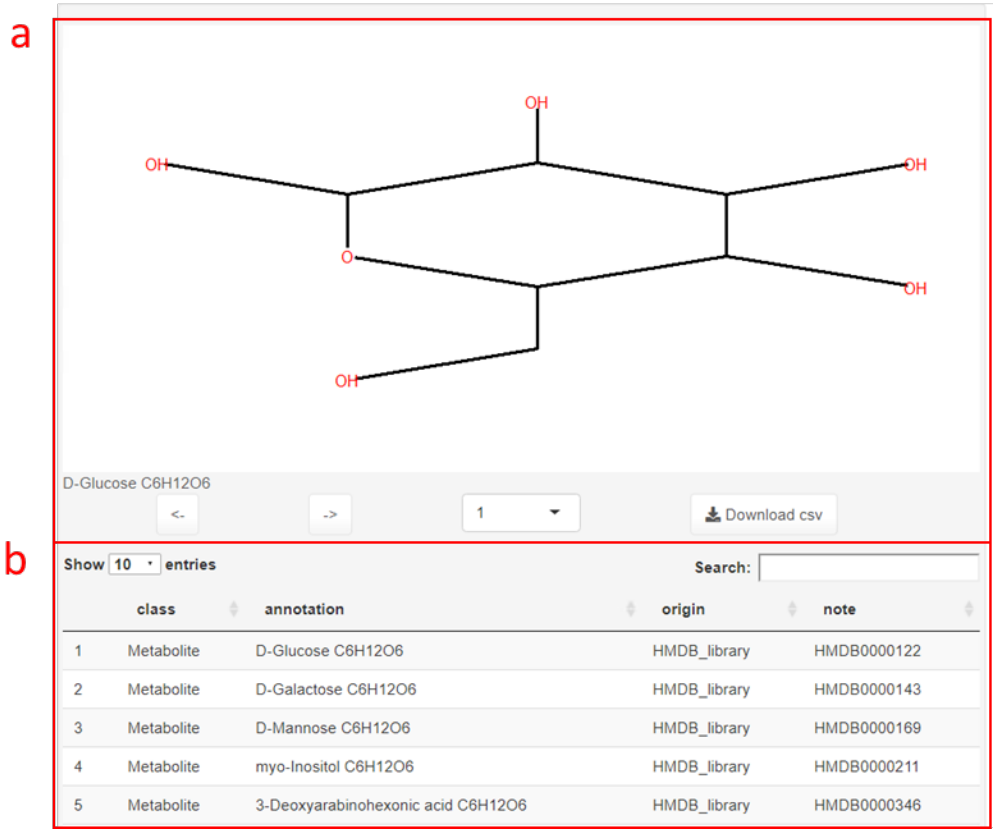


6. You can use the "Download plot" button to download a html webpage to visualize the network graph independent of the Shiny app, and the "Download csv" button to download the information of the nodes in the network. The download buttons will appear after hitting the plot button. Note: edits within the Shiny app will not go into the html file.

3.2.4 Possible structures exploration

A figure + data table is provided to explore structures of the selected node in the network graph.

1. The figure shows the chemical structure of the annotated metabolites. If the node is annotated as a putative metabolite, only the known parts of the putative metabolite will be shown.
   Scroll left or right, or select the entry number, to visualize different annotations. Right click and select to save image.
2. In the data table, class has 3 possible entries: Metabolite if it is documented in database such as HMDB library; Putative metabolite if it is transformed from a metabolite through a biotransformation edge; and Artifact if it is transformed by an abiotic edge. Use the download button to download the data table



4 Troubleshooting

4.1 Failing to install package `lc8`

Reinstall the packages `devtools` and `digest`.

4.2 Cannot find `cplexAPI` even if the installation seems successful

Check **R** version used in **RStudio** to see if `cplexAPI` is installed under the same R version library. Which R library `cplexAPI` goes to depends on the R path specified in `Environment Variables`.

4.3 Add **R** to `PATH`

1. Go to `Environment Variables`:
   search `PATH` in `windows` → open `edit Environment Variables` → `Environment Variables` or
   `control panel` → `system and security` → `System` → `Advanced system Settings` (on your left) → `Advanced` → `Environment Variables`
2. In the lower Panel select the `Path Variable` and select `Edit`, add the R path (`C:\Program Files\R\R-4.0.3\bin\x64`, if installed at default location) to the `Path Variable`.
3. You may need to restart computer for the R path to take effect.

4.4 Add **Rtools40** to `PATH`

1. Add the path `C:\Rtools\bin` to the `Path Variable`in `Environment Variables`
2. Run the line in **R**:
   `writeLines('PATH="${RTOOLS40_HOME}\\usr\\bin;${PATH}"', con = "~/.Renviron")`
   Use the line below in R console to check for successfully adding Rtools40
   `Sys.which("make")`
   Expected output: `## "C:\\rtools40\\usr\\bin\\make.exe`

Supplementary Note 4. NetID pseudocode

The whole algorithm runs in the following workflow:

1. Input data and data cleaning
2. Initializing and defining NodeSet and EdgeSet
3. Expanding candidate annotation through edge propagation
4. Defining CplexSet
5. Scoring candidate node and edge annotations
6. Global optimization
7. Network annotation
8. Output

Note: description following the "#" sign are comments, and will not be run by the code.

1. Input data and data cleaning

Input

Define *Mset* as a list, read in

Experimental MS1 data (containing mz, RT and intensity from LC-MS)
Experimental MS2 data (associated with MS1 data)
ionization mode
HMDB library file
HMDB library MS2 files (pos or neg)
Known library file (with curated RT)
Atom difference table (rule table for biotransformations, adducts, isotopes, etc.)

Remove background peaks and duplicated entries

For each peak

IF its intensity in procedure blank > 0.5-fold of that in biological samples

Remove the peak

For any two (or more) peaks,

IF their mz difference is within *mz_tol* **AND** RT difference within *rt_tol*

Create a new entry by merging multiple entries

Take the median *mz* and *RT* of entries as new *mz* and *RT*

Take the largest intensity value of all entries in each sample as sample intensity

Remove old duplicated entry peaks

2. Initializing and defining NodeSet and EdgeSet

NodeSet: Each peak is a node, and becomes an entry in nodeset.

Define *NodeSet* as a list,

For each *node* in *NodeSet*,

store one peak's *mz*, *RT*, *intensity*, *MS2*

Set up seed nodes

For each *node* in *NodeSet*,

IF *mz_difference* < 10 ppm by comparing measured mz to all formulae in HMDB library

Add HMDB ID, formula and class information to corresponding node

Adjust systematic measurement errors
For all nodes in *NodeSet* that has at least one HMDB entry,
Linear regression using measured mz values of selected nodes and their HMDB formula mz
an absolute mz adjustment factor $\varepsilon_{absolute}$ (independent of measured mz)
a relative mz adjustment factor $\varepsilon_{relative}$ (linearly dependent on measured mz)
For each node *u* in *NodeSet*
Recalculate measured mz by applying

$$u_{\mathrm{mz,adjusted}} = u_{\mathrm{mz,measured}} \times (1 + \varepsilon_{\mathrm{relative}}) + \varepsilon_{\mathrm{absolute}}$$

EdgeSet: Each edge connects two nodes by a mass difference defined in atom difference table
Define *EdgeSet* as a list,
For each pair of node *u* and *v* (assuming $v_{mz} > u_{mz}$), and for each difference D_i in atom difference table,
IF $|\left(v_{\mathrm{m/z}} - u_{\mathrm{m/z}}\right) - D_i| < v_{\mathrm{m/z}} \times 10\ \mathrm{ppm}$,
IF D_i is a Biotransformation connection (defined in atom difference table)
Add an edge with *node1* = *u*, *node2* = *v*, and related info for D_i to *EdgeSet*
IF D_i is an Abiotic connection (defined in atom difference table) **AND**
IF $|v_{\mathrm{RT}} - u_{\mathrm{RT}}| < 0.2\ \mathrm{min}$
Add an edge with *node1* = *u*, *node2* = *v*, and related info for D_i to *EdgeSet*.

EdgeSet expansion with additional abiotic connections (see manuscript methods)
including oligomers, multi-charge species, heterodimers, in-source fragments, etc.
For each pair of node *u* and *v*
IF $|v_{\mathrm{RT}} - u_{\mathrm{RT}}| < 0.2\ \mathrm{min}$ **AND**
IF properties of node u and v satisfy the criteria for additional abiotic connections
Add an edge with *node1* = *u*, *node2* = *v*, and related info for D_i to *EdgeSet*.

3. Expanding candidate node annotation through edge propagation
By applying the atom difference of edge (u, v) on the formula assigned to seed node u,
we can derive a new candidate formula for the connected node v.
Iterating the process to all candidate formulae of node u through edge (u, v) will
further expand candidate formulae for node v.
Seed nodes formulae from HMDB belong to Metabolite class.
For each *edge (u, v)* connecting node *u*, *v* in *EdgeSet* **AND** D_i is a Biotransformation connection
For each candidate formula of node *u*, u_{formula}, that belongs to Metabolite class
IF calculated mz of $u_{\mathrm{formula}} + D_{\mathrm{i,formula}}$ is within 5 ppm of measured mz of node *v*
Add combined formula ($u_{\mathrm{formula}} + D_{\mathrm{i,formula}}$) with Metabolite class to node *v*
For each *edge (u, v)* connecting node *u*, *v* in *EdgeSet* **AND** D_i is an Abiotic connection
For each candidate formula of node u, u_{formula}
IF calculated mz of $u_{\mathrm{formula}} + D_{\mathrm{i,formula}}$ is within 5 ppm of measured mz of node *v*
Add combined formula ($u_{\mathrm{formula}} + D_{\mathrm{i,formula}}$) with Artifact class to node *v*
REPEAT LINE 81-84 (above 4 lines) three times (three rounds of expansion via abiotic connections)
REPEAT LINE 77-85 (above 9 lines) two times (two rounds of expansion via biotransformation connections)

4. Defining CplexSet

CplexSet defines the network structure for global network optimization
Each node may contain zero, one or more than one candidate node annotations
Each candidate node annotation defines an ilp_node in CplexSet (ilp means integer linear programing)
We use *ilp_nodes* to score and record each candidate node annotation
Similarly, we use *ilp_edges* to score and record each candidate edge annotation

For each node *u* in *NodeSet*,
  For each candidate node annotation a_i in *u,*
    IF the combination of *node_id*, *formula* and *class* of a_i is not in *ilp_nodes*
      Add the candidate annotation (u, a_i) to *ilp_nodes*
For each edge (*u,v)* in *EdgeSet*,
  For each atom difference D_k
    For each candidate node annotation a_i in *u*, and b_j in *v*
      IF combined formula $(a_i + D_{\mathrm{k,formula}}) == b_j$
        Add the candidate edge annotation (u, v, a_i, b_j, D_k) to *ilp_edges*

5. Scoring candidate node and edge annotations

The scoring system is to assign high scores to annotations that effectively align the experimentally observed
ion peaks with prior metabolomics knowledge.
See manuscript method section for more details on score terms.

For each candidate node annotation (u, a_i), its score $S(u, a_i)$ is the sum of
  $\mathrm{S}_{\mathrm{m/z}}(u, a_i)$ # based on m/z accuracy,
  $\mathrm{S}_{\mathrm{RT}}(u, a_i)$ # based on RT of measured peaks and known standards
  $\mathrm{S}_{\mathrm{MS2}}(u, a_i)$ # based on MS2 of measured peaks and database MS2
  $\mathrm{S}_{\mathrm{database}}(u, a_i)$ # based on if the annotation a_i exists in HMDB
  $\mathrm{S}_{\mathrm{missing_{isotope}}}(u, a_i)$ # based on if the expected isotopic peak for a_i is missing
  $\mathrm{S}_{\mathrm{rule}}(u, a_i)$ # based on if a_i violates basic chemical rules
  $\mathrm{S}_{\mathrm{derivative}}(u, a_i)$ # based on if a_i is derived from a parent peak with a high annotation score

For each candidate node annotation (u, v, a_i, b_j, D_k), its score $S(u, v, a_i, b_j, D_k)$ is the sum of
  $\mathrm{S}_{\mathrm{MS2\_similarity}}(u, v, a_i, b_j, D_k)$ # based on similarity of measured MS2 spectra of node *u* and *v*
  $\mathrm{S}_{\mathrm{co\_elution}}(u, v, a_i, b_j, D_k)$ # based on RT difference of node *u* and *v*
  $\mathrm{S}_{\mathrm{type}}(u, v, a_i, b_j, D_k)$ # based on the connection type, defined by D_k in the atom difference table
  $\mathrm{S}_{\mathrm{isotope\_intensity}}(u, v, a_i, b_j, D_k)$ # based on the intensity ratio and expected natural abundance

6. Global optimization

The goal is to find annotations for each node so as to maximize the sum of the scores across the network
under the constraints that each node is assigned a single annotation,
and that the network annotation is consistent.
An example optimization problem using CPLEX in R can be found at
https://cran.r-project.org/web/packages/cplexAPI/vignettes/cplexAPI.pdf

Define *x* as a vector of binary number
  # if x_i = 1, the candidate node or edge annotation is selected in the global optimal network
  # if x_i = 0, then the annotation is not selected.

Length(x) = (number of candidate node annotation) + (number of candidate edge annotation)

Define *Obj* as a vector with the same length of x

Obj records score for each candidate node or edge annotation

Obj · x is the total scores of the network. Global optimization maximizes the total scores.

Obj = c(scores for ilp_nodes, scores for ilp_edges)

Constraints are defined as below.

For a sample constraint $a_1x_1 + a_2x_2 \leq b$,

$a_1x_1 + a_2x_2$ is the left-hand side, b is the right-hand side and "≤" is the sense of the constraint

$[a_1, a_2]$ is the constraint matrix, $[a_1, a_2]$ · x is the left-hand side of a constraint

Define *mat* as a matrix

mat · x is the left-hand side of the constraint.

mat is a sparse matrix as most number in *mat* are zero.

Column number of *mat* = Length(x)

Row number of *mat* = Number of constraints

Define *triplet_mat* as a matrix

we use triplet (*i,j,v*), i.e. the value (*v)* in the *i*th row, and *j*th column, to describe *mat*.

Column number of *triplet_mat* = 3

Row number of *triplet_mat* = number of non-zero entry in *mat*

Define *rhs* as a numeric vector

rhs is the right-hand side of a constraint

Length(*rhs*) = Number of constraints

Define *sense* as a character vector

rhs describes the signs between left- and right-hand sides

Signs includes less or equal (L), equal (E), greater or equal (G)

Length(*sense*) = Number of constraints

How constraint matrix is filled up is described below.

(I) Constrain each peak has single annotation.

Total number for this constraint = number of peaks

for all annotation a_i of peak u, sum (x_{ai}) = 1

For each candidate node annotation in *ilp_nodes*,

Add *i* = *peak_id*, *j* = *ilp_node_id*, *v* = 1 to *triplet_mat*

Add rep(1, number of peaks) to *rhs*

Add rep('E', number of peaks) to *sense*

(II) Constrain each edge annotation exists only if related candidate node annotations exist.

Total number for this constraint = number of candidate edge annotations * 2

In candidate edge annotation $e(u,v,a_i,b_j,D_k)$, $x_e - x_{ai} \leq 0$ and $x_e - x_{bj} \leq 0$

$i_{current}$ = total number of constraints from (I)

For each candidate edge annotation in *ilp_edges*,

$i_{current} = i_{current} + 1$

Add $i = i_{current}$, j = *ilp_edge_id,* and v = 1*;*

$i = i_{current}$, j = *ilp_node_id* for a_i, and v = -1 to *triplet_mat*

176 $i_{current}$ = $i_{current}$ + 1
177 **Add** *i* = $i_{current}$, *j* = *ilp_edge_id,* and *v* = 1;
178 *i* = $i_{current}$, *j* = *ilp_node_id* for b_j, and *v* = -1 to *triplet_mat*
179 **Add** rep(0, number of candidate edge annotations * 2) to *rhs*
180 **Add** rep('L', number of candidate edge annotations * 2) to *sense*
181
182 # (III) Constrain an isotope annotation exists only if the isotope connection exists
183 # Total number for this constraint = number of candidate edge annotation that is an isotope connection
184 # In candidate edge annotation e(u,v,a_i,b_j,D_k), assuming b_j is an isotope annotation, $x_e - x_{bj} = 0$
185 $i_{current}$ = total number of constraints from (I-II)
186 **For each** candidate edge annotation in *ilp_edges* that is an isotope connection
187 $i_{current}$ = $i_{current}$ + 1
188 **Add** *i* = $i_{current}$, *j* = *ilp_edge_id,* and *v* = 1;
189 *i* = $i_{current}$, *j* = *ilp_node_id* for b_j, and *v* = -1 to *triplet_mat*
190 **Add** rep(0, number of candidate edge annotation that is an isotope connection) to *rhs*
191 **Add** rep('E', number of candidate edge annotation that is an isotope connection) to *sense*
192
193 # (IV) Constrain only one edge can exist between two nodes
194 # Total number for this constraint = number of multiple-edge events * 2
195 # When multiple edges exist between two nodes, we call it multiple-edge event
196 # Assuming candidate edge annotation $e(u,v,a_i,b_j,D_k)$, $e'(u,v,a_i,b_j,D_k)$ and multiple edges exist
197 # At most one edge exist: $x_e + x_{e'} + \ldots - x_{ai} \leq 0$, $x_e + x_{e'} + \ldots - x_{bj} \leq 0$
198 $i_{current}$ = total number of constraints from (I-III)
199 **For each** multiple edge event
200 $i_{current}$ = $i_{current}$ + 1
201 **Add** *i* = $i_{current}$, *j* = *ilp_node_id* for a_i, and *v* = -1 to *triplet_mat*
202 **For each** candidate annotation *e* that exist between node u and node v with a_i and b_j annotation
203 **Add** *i* = $i_{current}$, *j* = *ilp_edge_id* for *e*, and *v* = 1 to *triplet_mat*
204 $i_{current}$ = $i_{current}$ + 1
205 **Add** *i* = $i_{current}$, *j* = *ilp_node_id* for b_j, and *v* = -1 to *triplet_mat*
206 **For each** candidate annotation *e* that exist between node u and node v with a_i and b_j annotation
207 **Add** *i* = $i_{current}$, *j* = *ilp_edge_id* for *e*, and *v* = 1 to *triplet_mat*
208 **Add** rep(0, number of multiple edge event * 2) to *rhs*
209 **Add** rep('L', number of multiple edge event * 2) to *sense*
210
211 # Pass parameters to CPLEX optimization
212 **Add** *CPLEX_para* = list(*nc* = Length(*x*), *nr* = number of constraint # number of columns and rows
213 *CPX_MAX*, # indicating maximization will be performed
214 *obj*, *rhs*, *sense*, # described above
215 *cnt*, *ind*, *val*, # describing mat in compressed sparse column (CSC) format
216 *lb* = 0, *ub* = 1, *ctype* = "B" # x's lower and upper bound, and its type is binary
217) to *CplexSet*
218
219 # CPLEX optimization

220 # *ilp_solution* contains a vector of binary number that
221 # denotes if a candidate node or edge annotation is selected for the global optimal network.
222 *ilp_solution* = Run_cplex(*CplexSet*)
223 *optimized_nodes* = Filter *ilp_nodes* that selected in *ilp_solution*
224 *optimized_edges* = Filter *ilp_edges* that selected in *ilp_solution*
225
226 **7. Network annotation**
227 # Seeds are node annotations that have direct annotations from HMDB,
228 **Define** *optimized_seed_nodes* = Filter *optimized_nodes* that have HMDB annotations
229 **Define** *optimized_nodes_M* = Filter *optimized_nodes* that are Metabolite class annotation
230 **Define** optimized_nodes_A = Filter *optimized_nodes* that are Artifact class annotation
231 **Define** *optimized_edges_M* = Filter *optimized_edges* that are Biotransformation connections
232 **Define** *optimized_edges_A* = Filter *optimized_edges* that are Abiotic connections
233
234 # The output network is an overlay of a biotransformation network and an abiotic network
235 **Define** *g_bio*, *g_abiotic*, *g_all* as graphs,
236 *g_bio* = graph (edges = *optimized_edges_M*,
237 nodes = optimized_nodes_M)
238 *g_abiotic* = graph (edges = *optimized_edges_A*,
239 nodes = *optimized_nodes* that exist in *optimized_edges_A*)
240 *g_all* = *g_bio* + *g_abiotic*
241
242 # Every node annotation in the network can trace back to seed annotation
243 **Define** *bio_dist* as a distance matrix,
244 # distance in row *i* and column *j* records
245 # the shortest distance from node *i* in *optimized_seed_nodes* to node *j* in optimized_nodes_M
246 *bio_dist* = shortest.paths(graph = *g_bio*,
247 from = *optimized_seed_nodes*,
248 to = optimized_nodes_M)
249 **Define** *abiotic_dist* as a distance matrix,
250 # distance in row *i* and column *j* records
251 # the shortest distance from node *i* in *optimized_nodes_M* to node *j* in optimized_nodes_A
252 *abiotic_dist* = shortest.paths(graph = *g_abiotic*,
253 from = *optimized_nodes_M*,
254 to = *optimized_nodes_A*)
255
256 # Path annotations to nodes
257 **For each** node *M* in *optimized_nodes_M*
258 **Find** seed node *H* that has shortest distances to *M* among all *optimized_seed_nodes* in *bio_dist*
259 **Define** path as the intermediate edges and nodes connecting from *H* to *M*
260 **Add** path annotation = c(HMDB name of *H*,
261 HMDB formula of *H*,
262 # Atom differences are specified by edge annotations in path
263 1st step atom difference, "->", intermediate node formula,

…

last step atom difference, "->", Formula of *M*) to node *M*

for acetyl-thiamine peak: "thiamine C12H16N4O1S1 + C2H2O1 -> C14H18N4O2S1"

For each node *A* in optimized_nodes_A

Find Metabolite class node *M* that has shortest distances to *A* among all in *abiotic_dist*

Define *path* as the intermediate edges and nodes connecting from *M* to *A*

Add *path annotation* = c(Formula of *M*,

Atom differences are specified by edge annotations in path

1st step atom difference, "->", intermediate node formula,

…

last step atom difference, "->", Formula of *A*) to node *A*

for glutamate sodium acetate adduct peak:

"C5H9N1O4 + Na1H-1 -> C5H8Na1N1O4 + C2H4O2 -> C7H12N1Na1O6"

8. Output

csv format

For all peaks,

compiles peak_id, medMz, medRt, log10_inten, class, formula, ppm_error, path annotation

Exports as NetID_output.csv

Shiny R visualization

Save all information as NetID_output.RData for Shiny R visualization