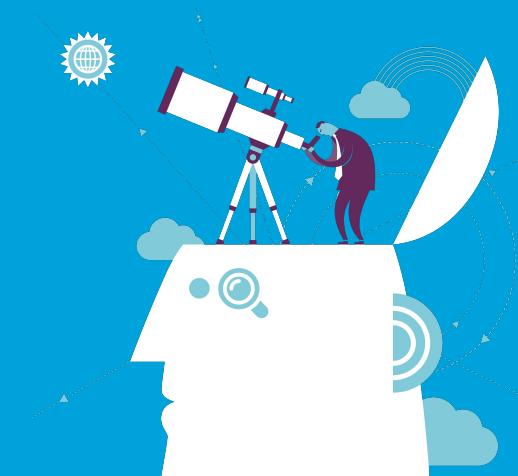


Multi-Omics Integration

Denise Slenter

ORCID: 0000-0001-8449-1318

2024-05-30 Nutriome Workshop 1



Four main types of pathway analysis

Over-Representation Analysis (ORA)

Functional Class Scoring (FCS)

Pathway Topology Analysis (TPA)

Network Enrichment Analysis (NEA)

Four main types of pathway analysis

Over-Representation Analysis (ORA)

Compares the overlap between metabolites of interest, metabolites present in a pathway, and the total number of metabolites measured and identified in a sample. This method does not include the arrangement of the elements in a PWM (topology), nor the ranking of the metabolites of interest.

Functional Class Scoring (FCS)

Pathway Topology Analysis (TPA)

Network Enrichment Analysis (NEA)

Four main types of pathway analysis

Over-Representation Analysis (ORA)

Compares the overlap between metabolites of interest, metabolites present in a pathway, and the total number of metabolites measured and identified in a sample. This method does not include the arrangement of the elements in a PWM (topology), nor the ranking of the metabolites of interest.

Functional Class Scoring (FCS)

Ranks the metabolites according to a statistical variable (e.g. p-value) and then compares the overlap between metabolites of interest to the ones present in a PWM. Topology is not considered in this approach.

Pathway Topology Analysis (TPA)

Network Enrichment Analysis (NEA)

Four main types of pathway analysis

Over-Representation Analysis (ORA)

Compares the overlap between metabolites of interest, metabolites present in a pathway, and the total number of metabolites measured and identified in a sample. This method does not include the arrangement of the elements in a PWM (topology), nor the ranking of the metabolites of interest.

Functional Class Scoring (FCS)

Ranks the metabolites according to a statistical variable (e.g. p-value) and then compares the overlap between metabolites of interest to the ones present in a PWM. Topology is not considered in this approach.

Pathway Topology Analysis (TPA)

The connections between individual metabolites are considered to estimate how a change in one particular metabolite might alter the complete pathway. This type of analysis depends on the relationships within a pathway

Network Enrichment Analysis (NEA)

Four main types of pathway analysis

Over-Representation Analysis (ORA)

Compares the overlap between metabolites of interest, metabolites present in a pathway, and the total number of metabolites measured and identified in a sample. This method does not include the arrangement of the elements in a PWM (topology), nor the ranking of the metabolites of interest.

Functional Class Scoring (FCS)

Ranks the metabolites according to a statistical variable (e.g. p-value) and then compares the overlap between metabolites of interest to the ones present in a PWM. Topology is not considered in this approach.

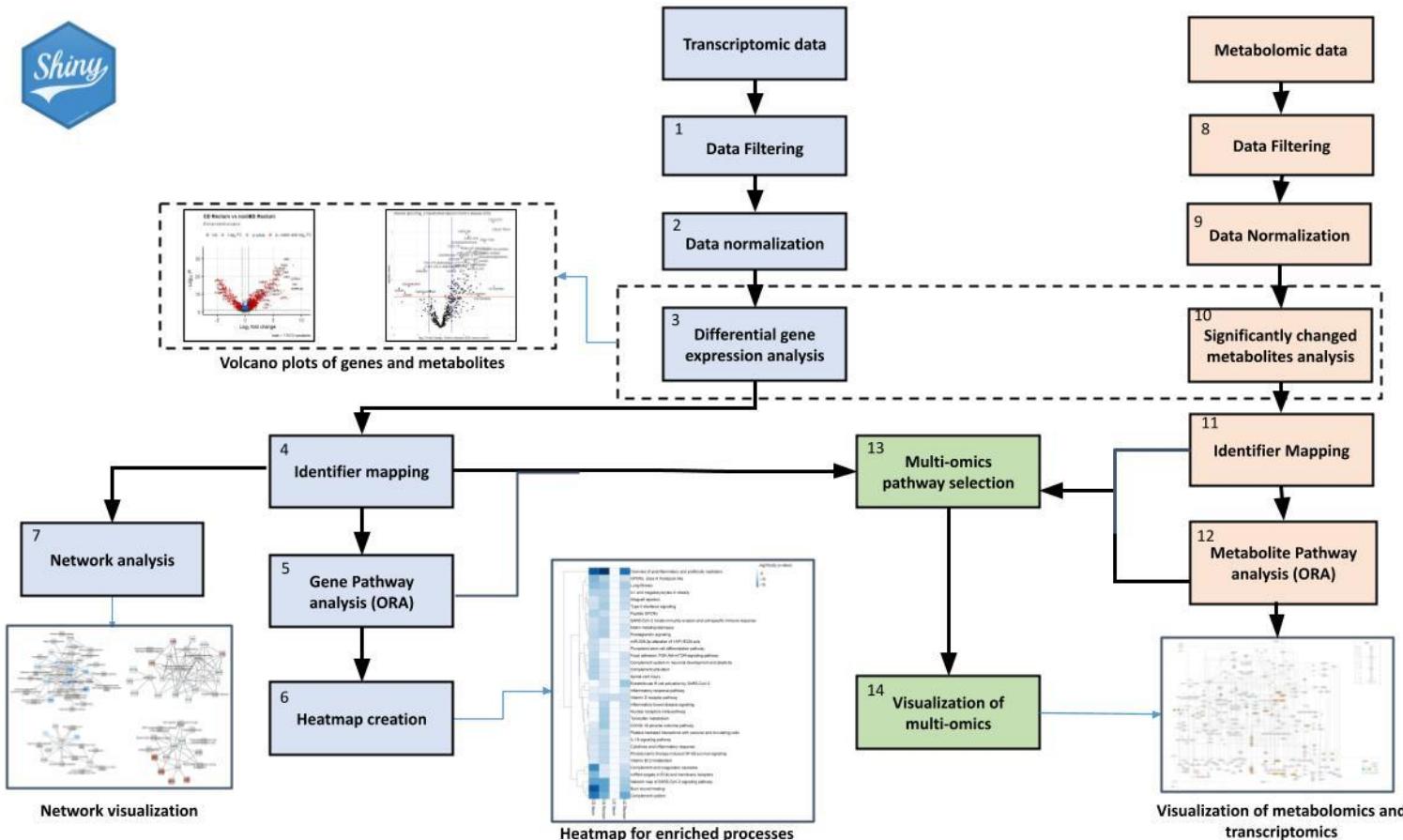
Pathway Topology Analysis (TPA)

The connections between individual metabolites are considered to estimate how a change in one particular metabolite might alter the complete pathway. This type of analysis depends on the relationships within a pathway

Network Enrichment Analysis (NEA)

This method surpasses the boundaries of a PWM, by comparing all relationships present in a chemical reaction network for overlap between metabolites of interest and metabolites present in the network.

Workflow transcriptomics and metabolomics integration



Transcriptomics results IBD study

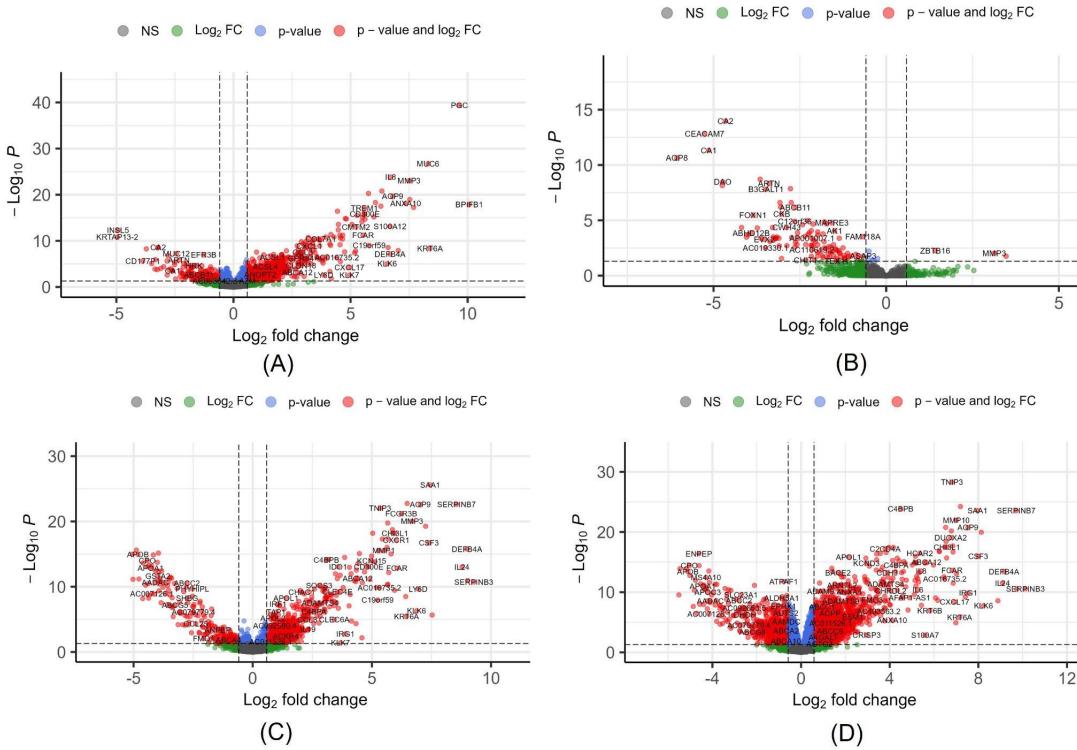


Figure 3. Volcano plots for differentially expressed genes in the ileum and rectum. (A) Crohn's disease patients versus controls on the ileum (B) Ulcerative colitis patients versus controls on the ileum (C) Crohn's disease patients versus controls on the rectum (D) Ulcerative colitis patients versus controls on the rectum. CD = Crohn's disease, UC = Ulcerative Colitis, non-IBD = healthy controls. The x-axis represents log2FC and the y-axis represents the corresponding significance given by $-\log_{10}(p\text{-value})$. 17,670 genes were analyzed.

Transcriptomics results IBD study

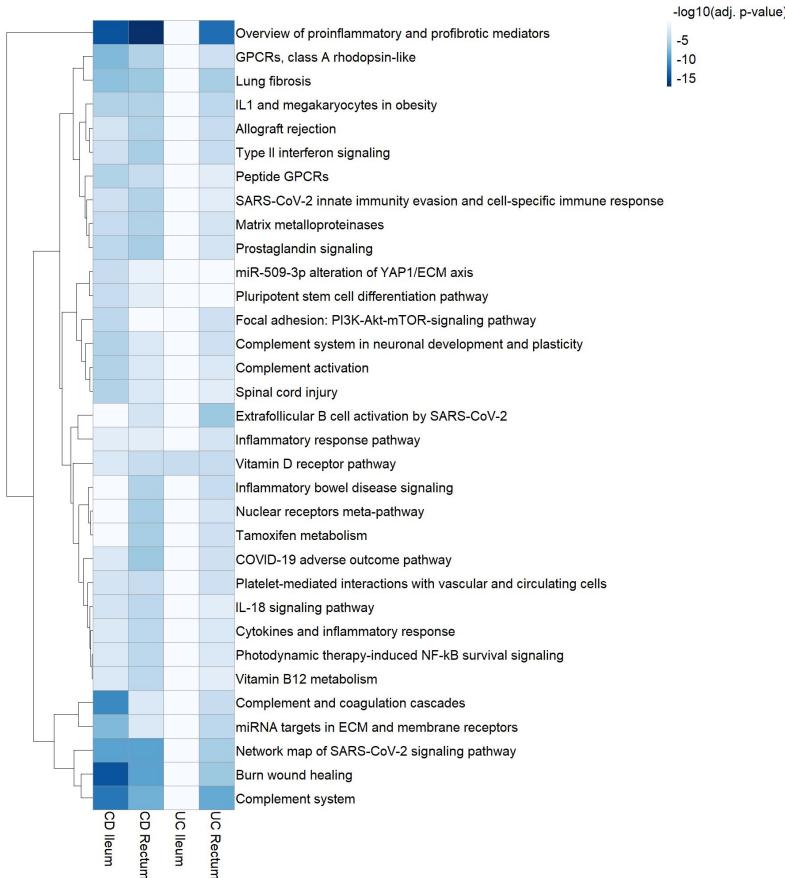


Figure 4. Comparison of altered pathways in Crohn's disease and ulcerative colitis on both the rectum and ileum samples. Rows represent enriched pathways while columns represent comparison pairs including disease and the biopsy location. Dark blue indicates a more significant pathway while light blue represents a less significant pathway.

Metabolomics results IBD study

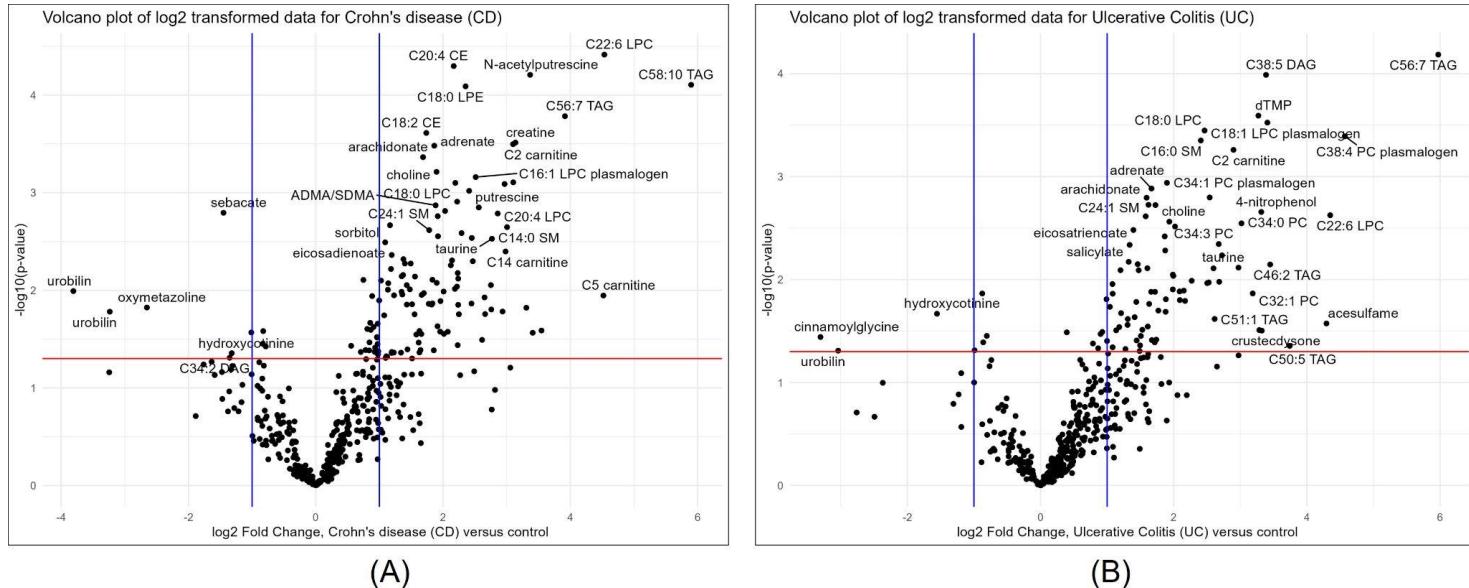


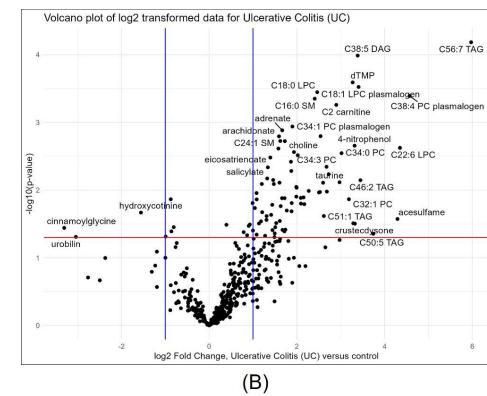
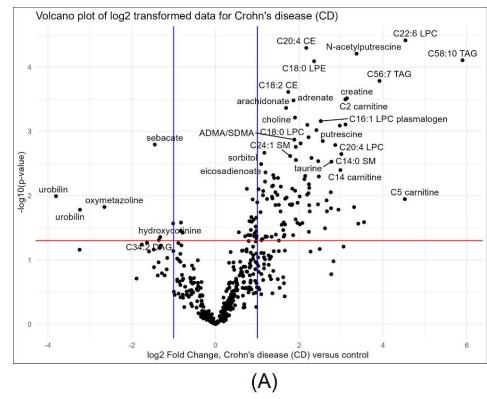
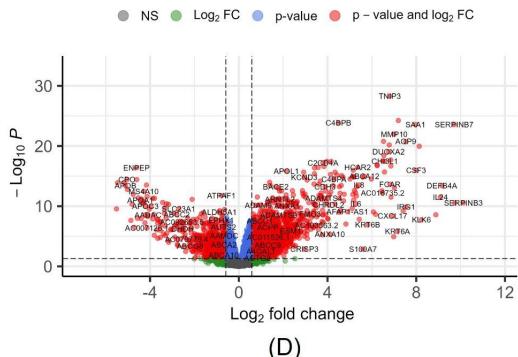
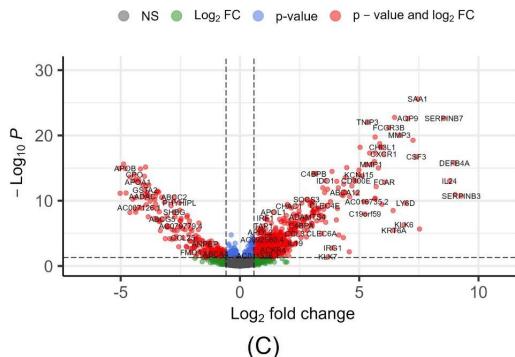
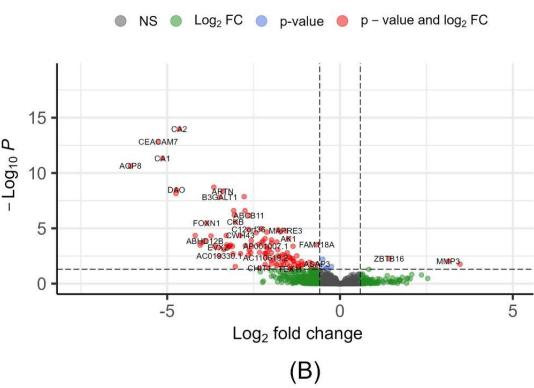
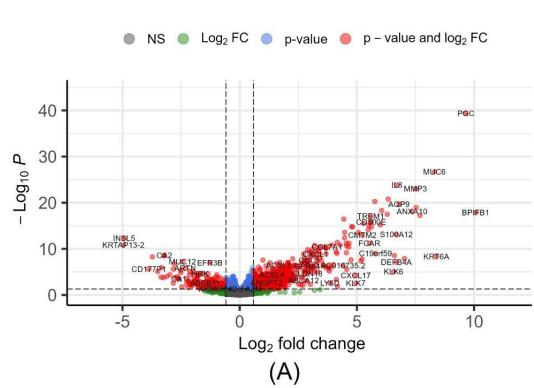
Figure 7. Volcano plots representing altered metabolites in the stool of IBD patients, with the vertical axis depicting the log2FC (blue line showing the threshold absolute log2Fold change ≥ 1), versus the y-axis showing the $-\log_{10}(p\text{-value})$ (red line depicting the p-value ≤ 0.05). (A) control versus Crohn's disease (CD) patients, (B) control group versus Ulcerative colitis (UC) patients.

Metabolomics results IBD study

Table 3. Pathway Analysis results for pathways containing 4 or more significantly changed metabolites in Crohn's disease (CD) and ulcerative colitis (UC). Overlapping pathways between both disorders are highlighted with a #, significant p-values (≤ 0.05) with a *.

Disorder	Pathway ID	Pathway Title	# Sign. Metabolites in PW	P-value	# Proteins in PW
CD	WP3925	Amino acid metabolism	8	0.0000*	91
	WP2525	Trans-sulfuration, one-carbon metabolism, and related pathways*	8	0.0468*	67
	WP4723	Omega-3 / omega-6 fatty acid synthesis*	7	0.1263	15
	WP15	Selenium micronutrient network*	6	0.0000*	86
	WP661	Glucose homeostasis	6	0.1695	1
	WP4726	Sphingolipid metabolism: integrated pathway*	5	0.0008*	26
	WP3953	mRNA, protein, and metabolite induction pathway by cyclosporin A	5	0.1353	7
	WP550	Biogenic amine synthesis	5	0.1844	15
	WP5176	Disorders of bile acid synthesis and biliary transport	4	0.0109	20
	WP706	Sudden infant death syndrome (SIDS) susceptibility pathways	4	0.2163	159
UC	WP4723	Omega-3 / omega-6 fatty acid synthesis*	6	0.1777	15
	WP15	Selenium micronutrient network*	5	0.0002*	86
	WP4726	Sphingolipid metabolism: integrated pathway*	4	0.0075*	26
	WP2525	Trans-sulfuration, one-carbon metabolism, and related pathways*	4	0.0238*	67

Transcriptomics and Metabolomics differences



Transcriptomics and Metabolomics differences

Size:

- Genome: 3.055 billion nucleic acid pairs within DNA
- Epigenome: high-throughput techniques covers less than 2% of the genomic sites where methylation occurs
 - From each gene several transcripts may arise, which can be categorized as (protein)-coding and (long and short) non-coding RNA
- Proteomics: minimum of 70.000 proteoforms to 1.5 million
- (endogenous) metabolome: into the thousands for vertebrates and tens or hundreds of thousands for plants
- Fluxomics: ??? combination of all metabolic reactions possible, as well as their involved enzyme kinetics.

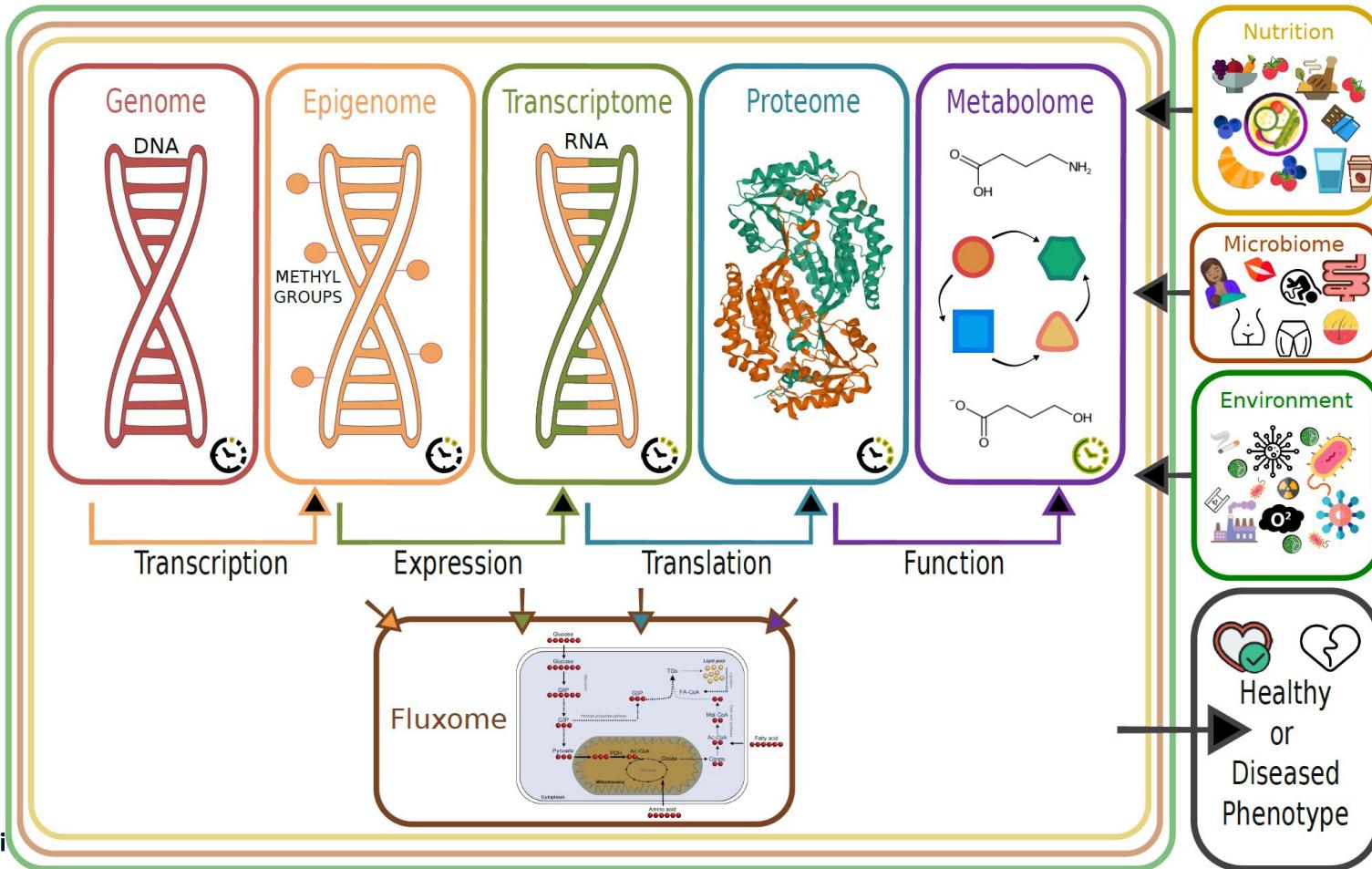
Dynamics

Tissue (distribution)

Model (organism)



Transcriptomics and Metabolomics differences



Multi-Omics integration

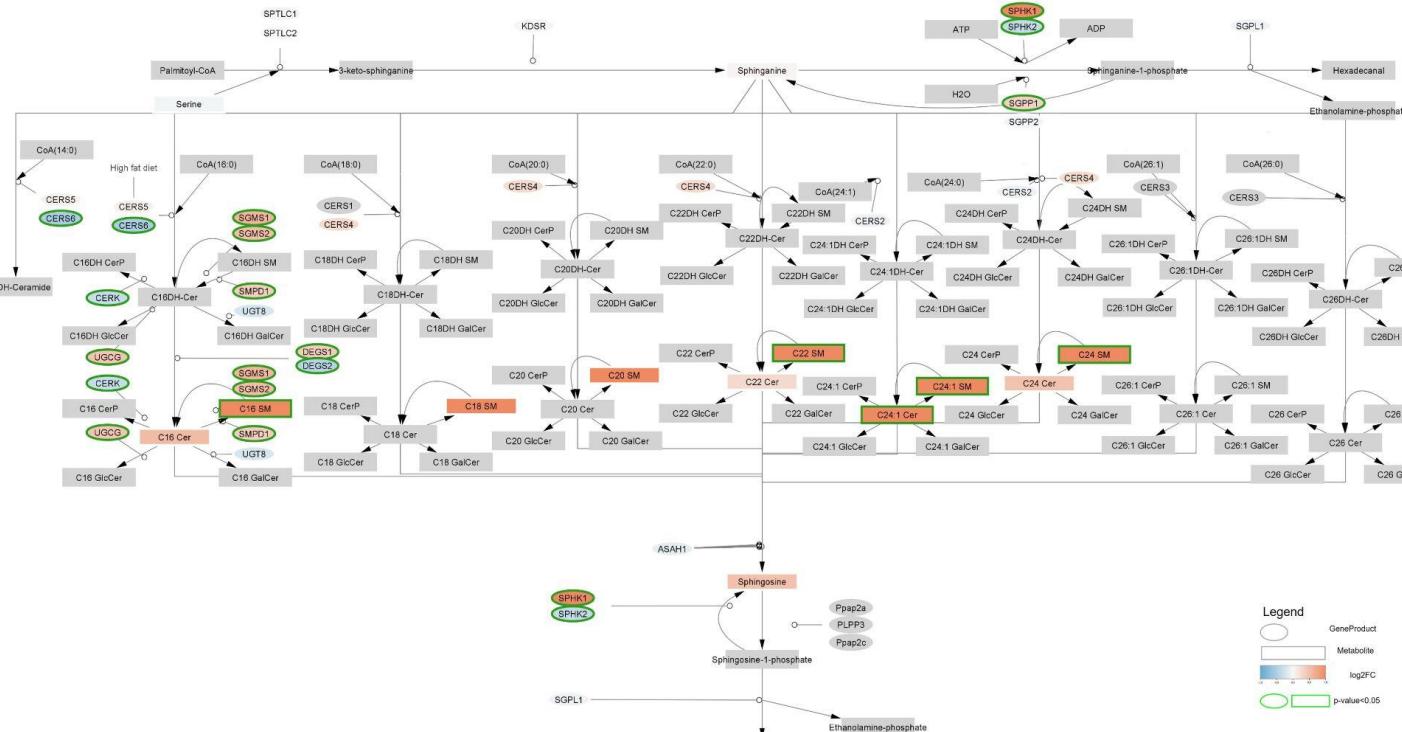


Figure 8. Sphingolipid metabolism: integrated pathway, <https://www.wikipathways.org/instance/WP4726>. The log2FC is indicated by a color gradient from blue (down-regulated) over white to red (up-regulated) on the nodes, significance ($p\text{-value} < 0.05$) is represented by a light-green border and omics data type is shown with a rectangle (metabolomics) and an ellipse (transcriptomics) shape. Nodes without measurement in the dataset are colored gray.

Discovering life's directed metabolic (sub)paths to interpret biochemical markers using the DSMN

Denise Slenter, Martina Kutmon, Chris Evelo, Egon Willighagen



Four main types of pathway analysis

Over-Representation Analysis (ORA)

Compares the overlap between metabolites of interest, metabolites present in a pathway, and the total number of metabolites measured and identified in a sample. This method does not include the arrangement of the elements in a PWM (topology), nor the ranking of the metabolites of interest.

Functional Class Scoring (FCS)

Ranks the metabolites according to a statistical variable (e.g. p-value) and then compares the overlap between metabolites of interest to the ones present in a PWM. Topology is not considered in this approach.

Pathway Topology Analysis (TPA)

The connections between individual metabolites are considered to estimate how a change in one particular metabolite might alter the complete pathway. This type of analysis depends on the relationships within a pathway

Network Enrichment Analysis (NEA)

This method surpasses the boundaries of a PWM, by comparing all relationships present in a chemical reaction network for overlap between metabolites of interest and metabolites present in the network.

Could solve: Low amount of data

Network model (or: graph)

Merging information from different resources into one larger network could aid in understanding metabolic changes which affect multiple processes.

These networks are also known as **graphs**, which entail the mathematical representation of a network.

Network model (or: graph)

Merging information from different resources into one larger network could aid in understanding metabolic changes which affect multiple processes.

These networks are also known as **graphs**, which entail the mathematical representation of a network.

Biochemical **graphs** are often modeled as [1]:

- Hypergraphs:
- Compound graph:
- Bipartite graphs:

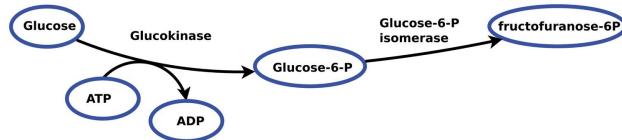
Network model (or: graph)

Merging information from different resources into one larger network could aid in understanding metabolic changes which affect multiple processes.

These networks are also known as **graphs**, which entail the mathematical representation of a network.

Biochemical **graphs** are often modeled as [1]:

- Hypergraphs: works well for visualizations, however, is not suitable for many graph algorithms
- Compound graph:
- Bipartite graphs:



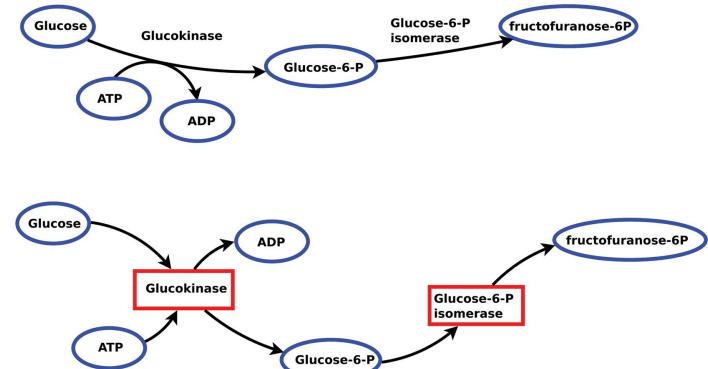
Network model (or: graph)

Merging information from different resources into one larger network could aid in understanding metabolic changes which affect multiple processes.

These networks are also known as **graphs**, which entail the mathematical representation of a network.

Biochemical **graphs** are often modeled as [1]:

- Hypergraphs: works well for visualizations, however, is not suitable for many graph algorithms
- Compound graph: can create a large number of edges influencing graph connectivity resulting in poor graph algorithm performance
- Bipartite graphs:



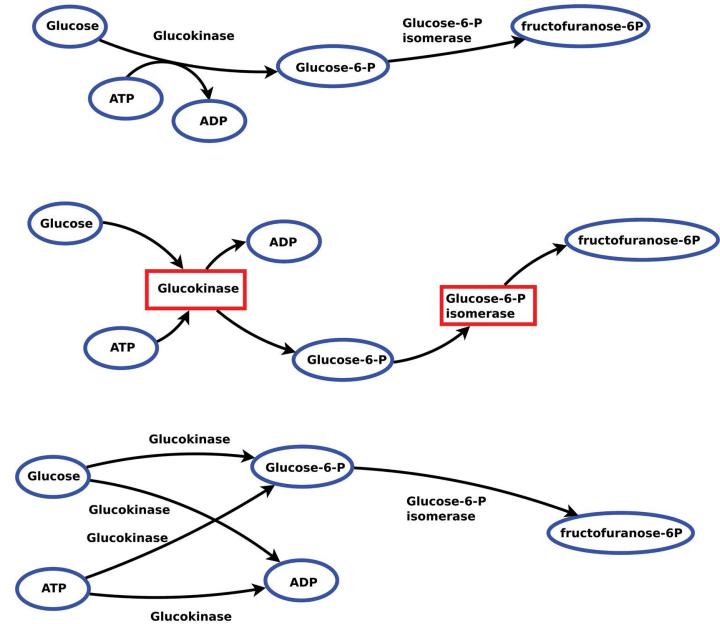
Network model (or: graph)

Merging information from different resources into one larger network could aid in understanding metabolic changes which affect multiple processes.

These networks are also known as **graphs**, which entail the mathematical representation of a network.

Biochemical **graphs** are often modeled as [1]:

- Hypergraphs: works well for visualizations, however, is not suitable for many graph algorithms
- Compound graph: can create a large number of edges influencing graph connectivity resulting in poor graph algorithm performance
- Bipartite graphs: can create paths that are biologically irrelevant between compounds of interest



[1] Frainay and Jourdan. (2017), DOI: [10.1093/bib/bbv115](https://doi.org/10.1093/bib/bbv115)

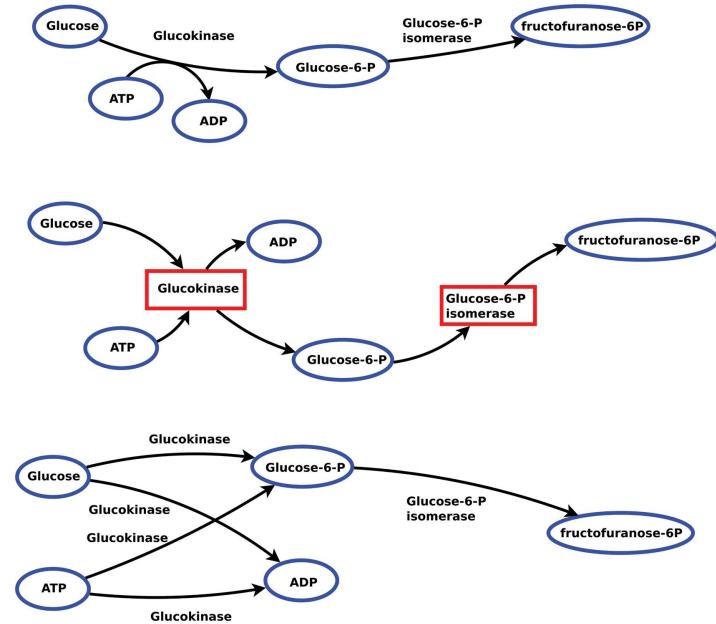
Network model (or: graph)

Merging information from different resources into one larger network could aid in understanding metabolic changes which affect multiple processes.

These networks are also known as **graphs**, which entail the mathematical representation of a network.

Biochemical **graphs** are often modeled as [1]:

- Hypergraphs: works well for visualizations, however, is not suitable for many graph algorithms
- Compound graph: can create a large number of edges influencing graph connectivity resulting in poor graph algorithm performance
- Bipartite graphs: can create paths that are biologically irrelevant between compounds of interest

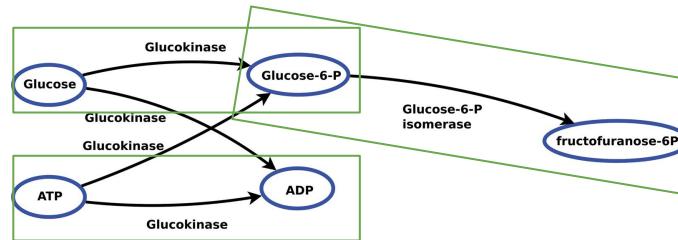


[1] Frainay and Jourdan. (2017), DOI: [10.1093/bib/bbv115](https://doi.org/10.1093/bib/bbv115)

Bipartite graphs issue and solutions

Issue:

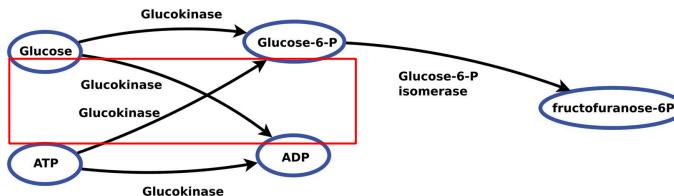
Creates paths that are biologically irrelevant between compounds of interest



Bipartite graphs issue and solutions

Issue:

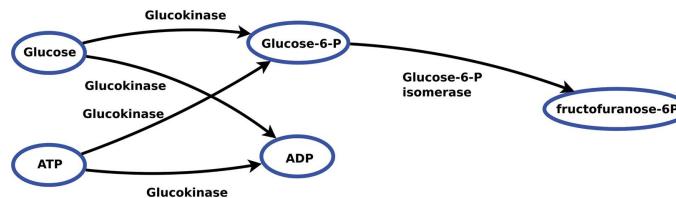
Creates paths that are biologically irrelevant between compounds of interest



Bipartite graphs issue and solutions

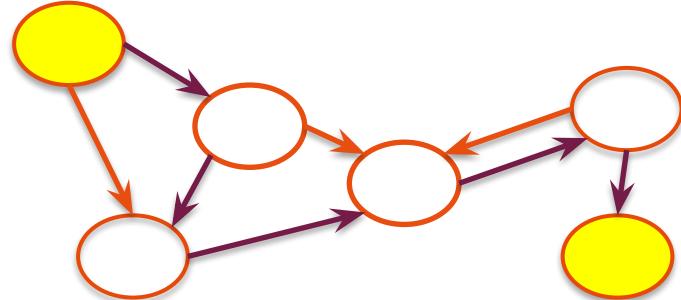
Issue:

Creates paths that are biologically irrelevant between compounds of interest

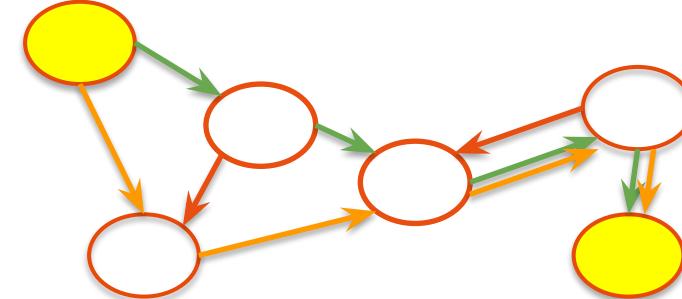


Solutions:

1. Reducing the number of calculated paths computationally (e.g. shortest path)



Steps:
5

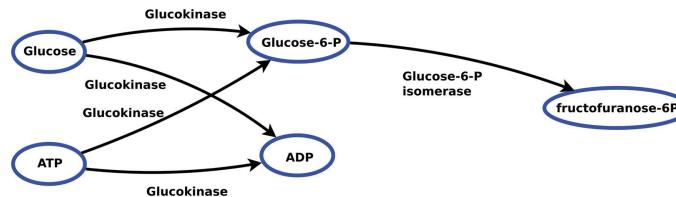


Steps:
4

Bipartite graphs issue and solutions

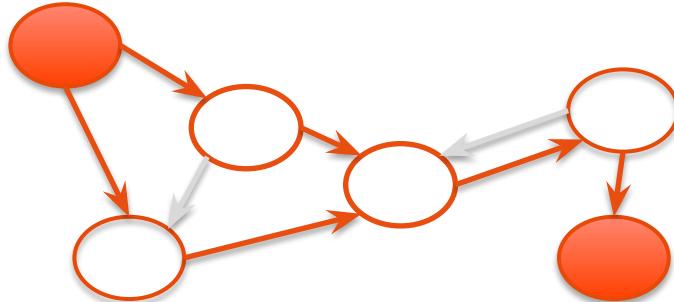
Issue:

Creates paths that are biologically irrelevant between compounds of interest



Solutions:

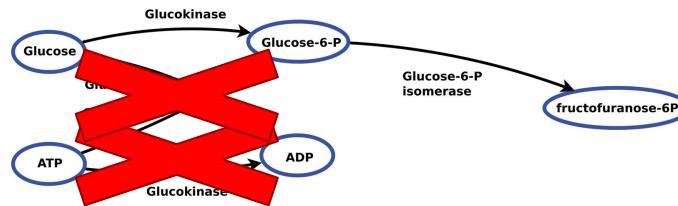
1. Reducing the number of calculated paths computationally (e.g. shortest path)



Bipartite graphs issue and solutions

Issue:

Creates paths that are biologically irrelevant between compounds of interest



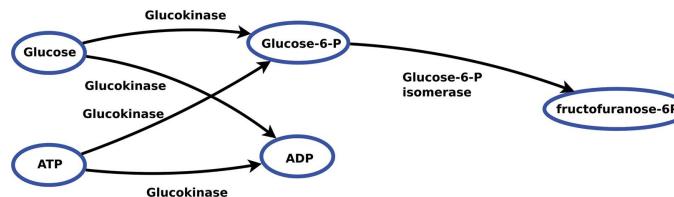
Solutions:

1. Reducing the number of calculated paths computationally (e.g. shortest path)
2. Exclude metabolites used in many reactions (e.g. energy carrier; proton donor, or acceptor)

Bipartite graphs issue and solutions

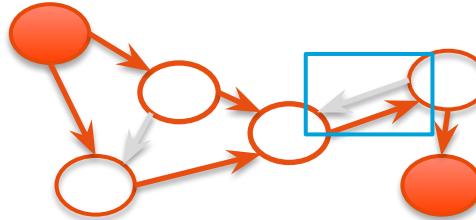
Issue:

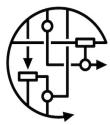
Creates paths that are biologically irrelevant between compounds of interest



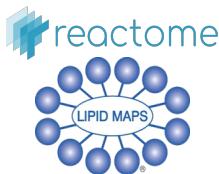
Solutions:

1. Reducing the number of calculated paths computationally (e.g. shortest path)
2. Exclude metabolites used in many reactions (e.g. energy carrier; proton donor, or acceptor)
3. Include directionality of reactions





WikiPathways
Pathways for the People



Metabolic Reaction Harmonization and Data Retrieval

Directed metabolic conversions
from three pathway knowledge
bases on human data

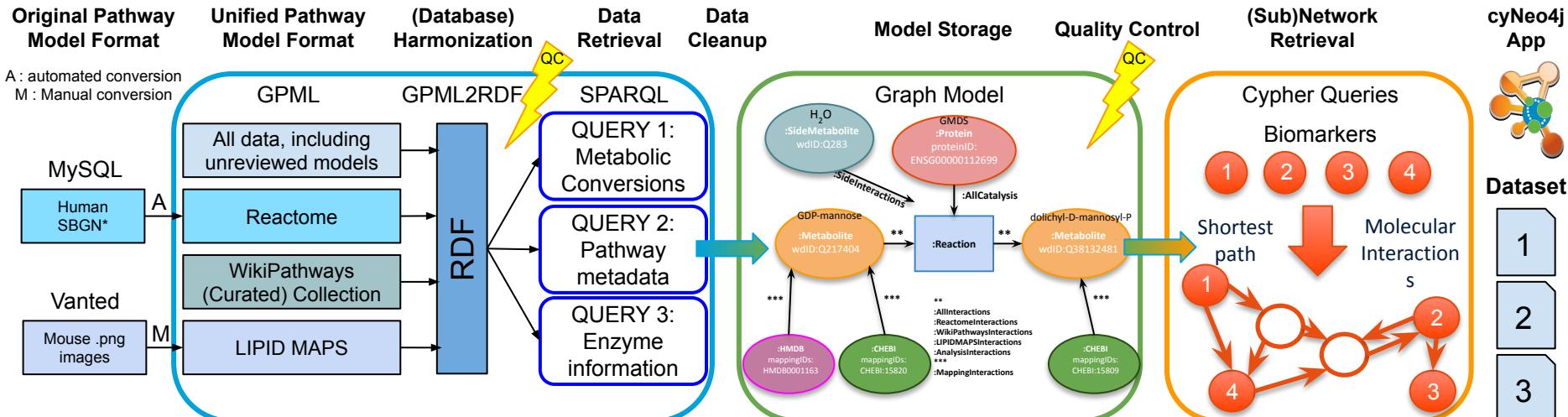


DSMN Database

Store (un)weighted directed interactions
Cypher Query Execution Planning
Several Algorithms (e.g. shortest-path)

Biomarker Visualization

Omics data visualization
Network extendable
Automatable (REST-API)



WikiPathways: [wikipathways.org](https://www.wikipathways.org), Slenter et al. (2018), DOI: [10.1093/nar/gkx1064](https://doi.org/10.1093/nar/gkx1064); Reactome: reactome.org, Fabregat et al. (2017), DOI: [10.1186/s12859-017-1559-2](https://doi.org/10.1186/s12859-017-1559-2); LIPID MAPS: lipidmaps.org

O'Donnell et al. (2019), DOI: [10.1126/scisignal.aaw2964](https://doi.org/10.1126/scisignal.aaw2964); SPARQL endpoint/RDF: sparql.wikipathways.org, Waagmeester et al. (2016) DOI: [10.1371/journal.pcbi.1004989](https://doi.org/10.1371/journal.pcbi.1004989)

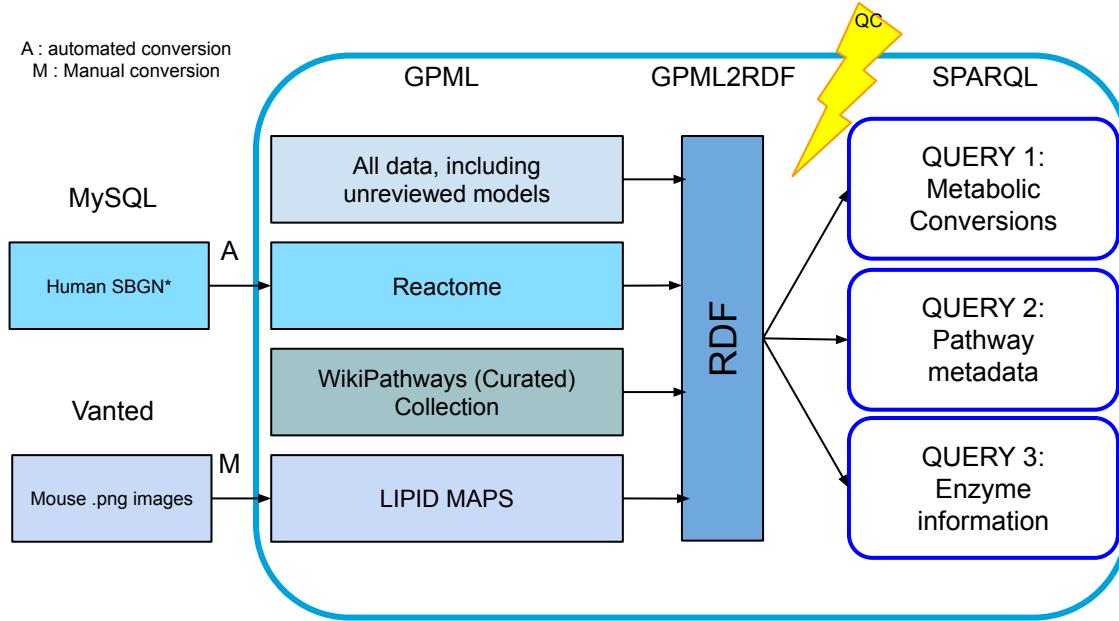
Neo4j: neo4j.com; Cytoscape: cytoscape.org, Shannon et al. (2003) DOI: [10.1101/gr.123930](https://doi.org/10.1101/gr.123930)



Maastricht University

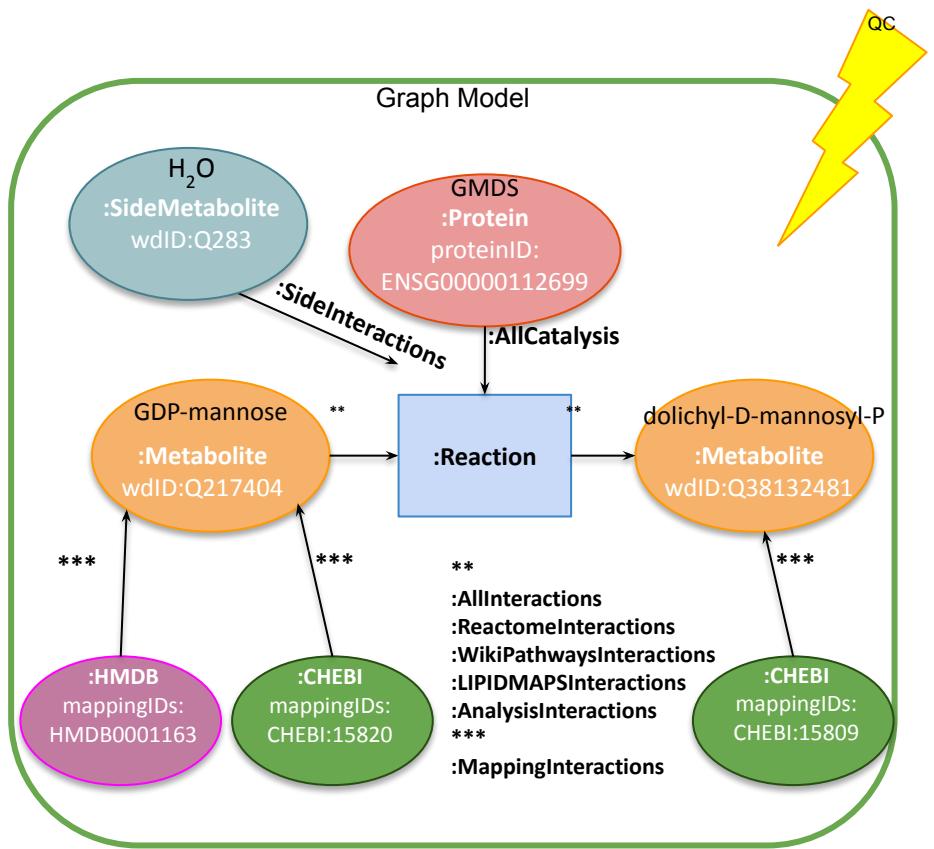
Original Pathway Model Format Unified Pathway Model Format (Database) Harmonization Data Retrieval

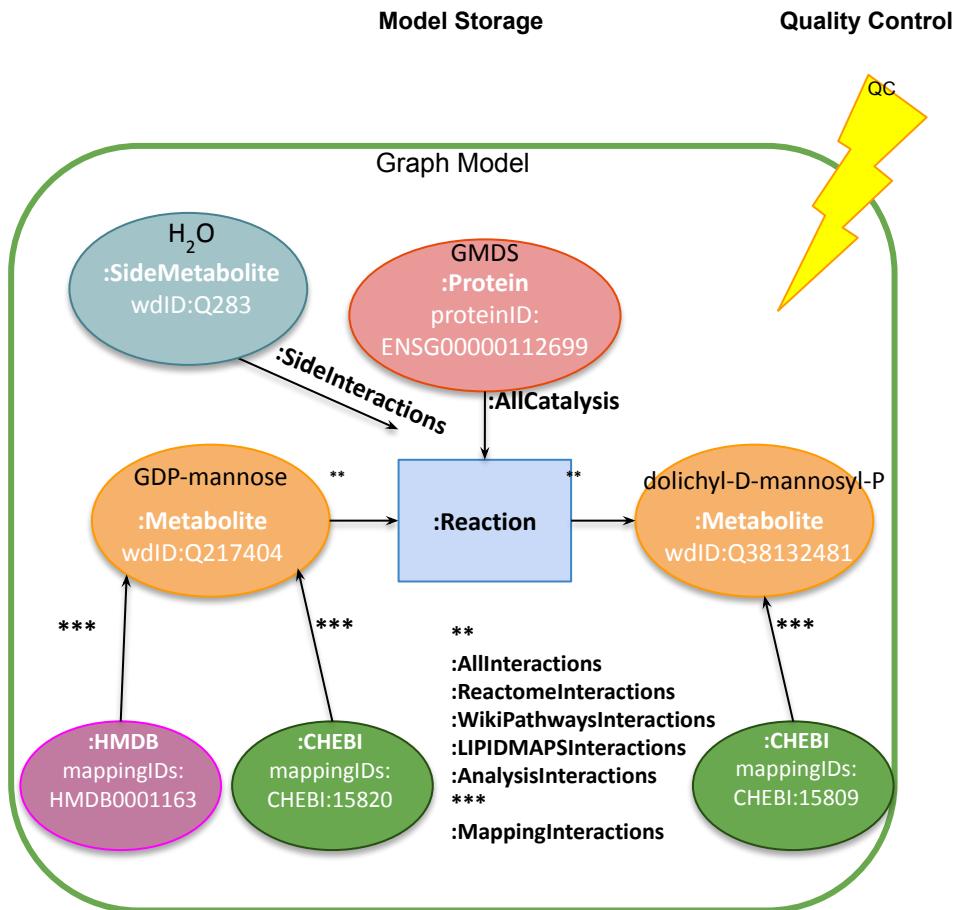
A : automated conversion
M : Manual conversion



Model Storage

Quality Control





Final DSMN graph database contained:

- 16.618 nodes
- 34.703 edges

Nodes:

- Metabolites: 2.397
- Enzymes: 999
- ChEBI Mappings: 2.204
- HMDB Mappings: 1.370

Edges:

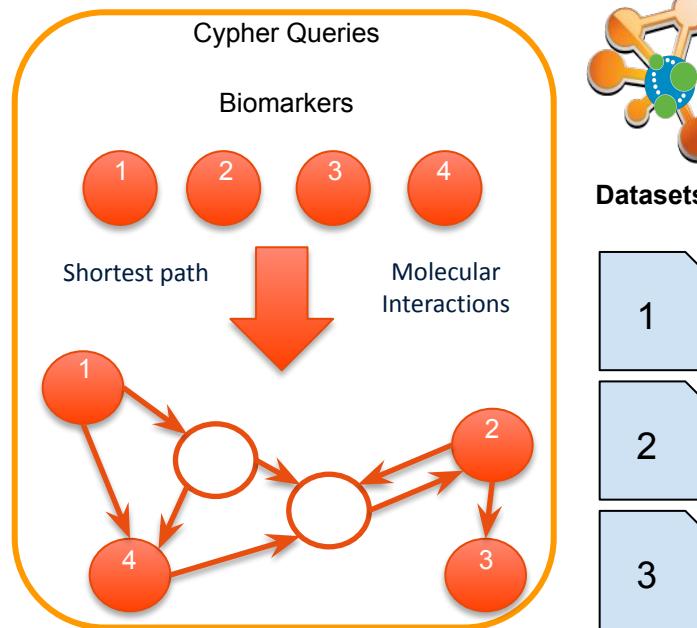
- 4358 metabolic reactions (:AllInteractions)
- 2615 (:WikiPathwaysInteractions) aka curated
- 2009 (:ReactomeInteractions)
- 379 (:LIPIDMAPSInteractions)
- 4209 (:AnalysisInteractions)

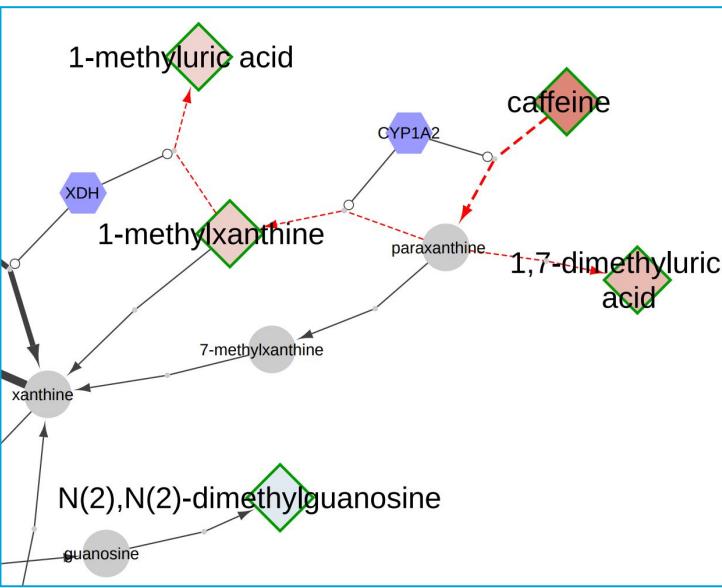
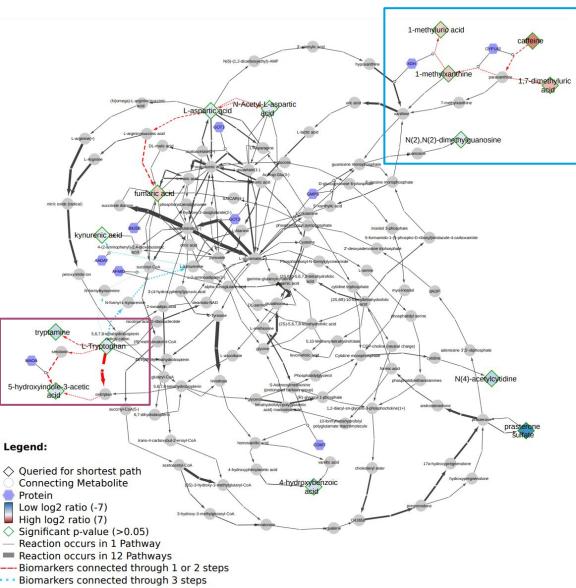
Side Metabolites:

- 212 IDs
- 254 edges relabeled (:SideInteractions)

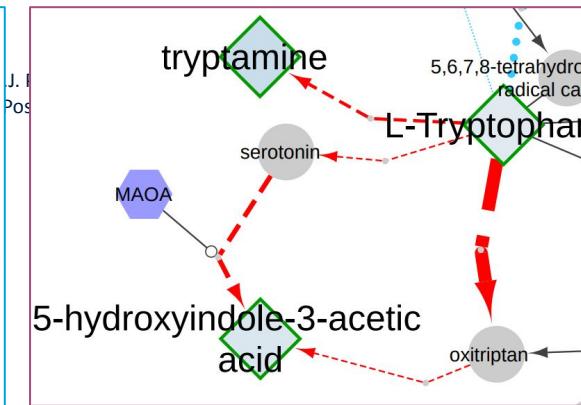
(Sub)Network Retrieval

cyNeo4j
App





"These metabolites cannot be regarded as individual variables in modeling approaches"



"Oxitriptan is an immediate precursor known to decline with age"

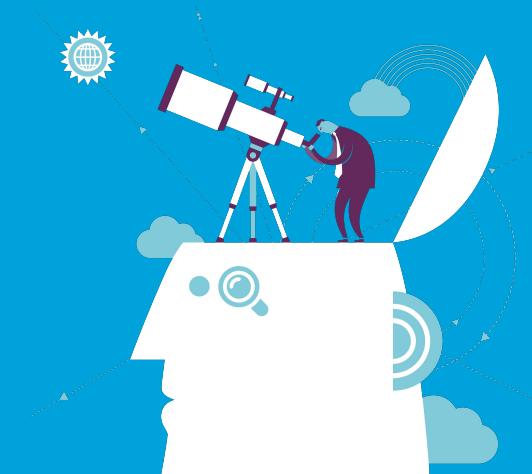
R Peters. "Ageing and the brain". In: Postgraduate Medical Journal 82.964 (Feb. 2006), pp. 84–88.

Community curation of kinetic data to support metabolic models through semantic web technology

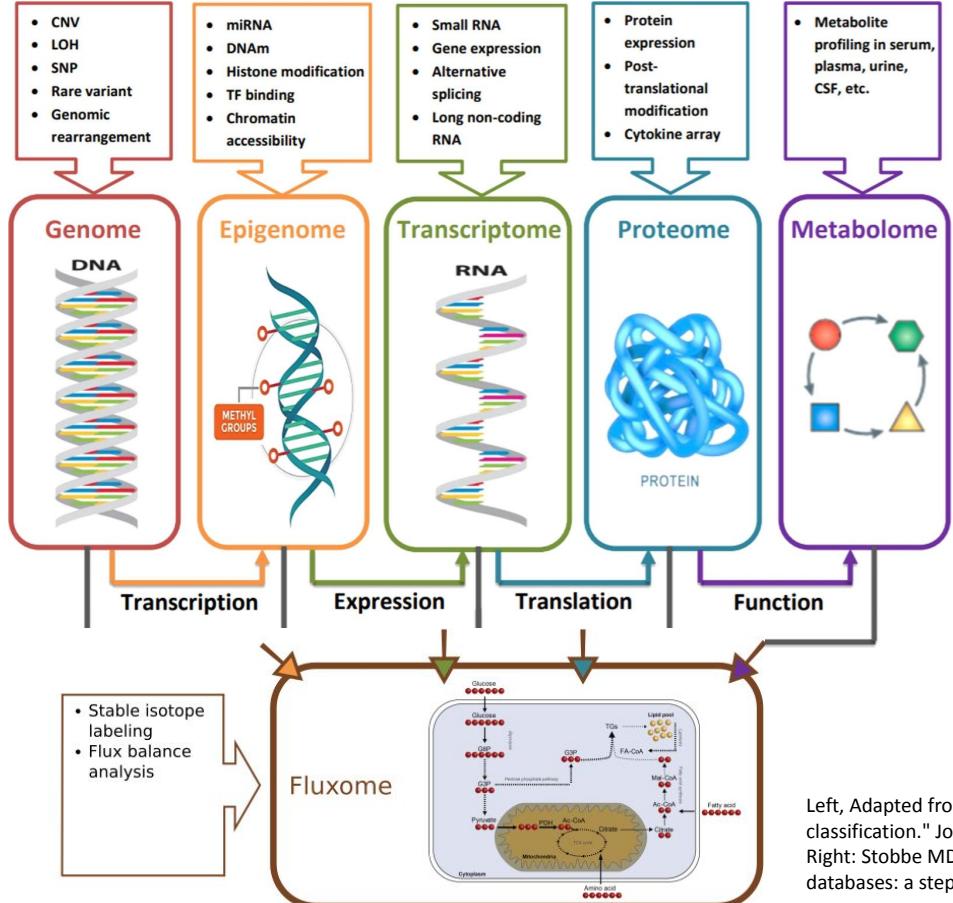
Denise Slenter, Egon Willighagen

Data and scripts:

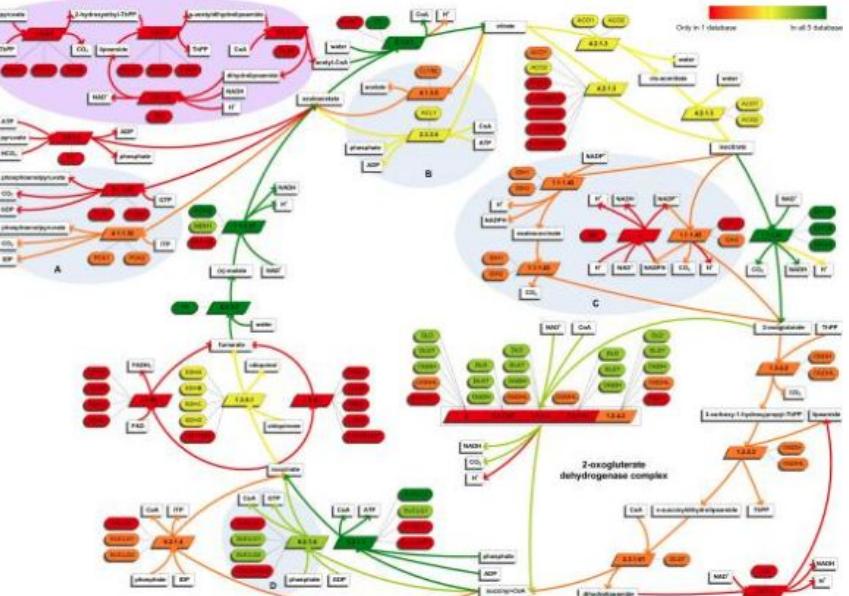
github.com/BiGCAT-UM/KinRDF



Different data types in OMICS research



INCOMPLETE PATHWAY DATA

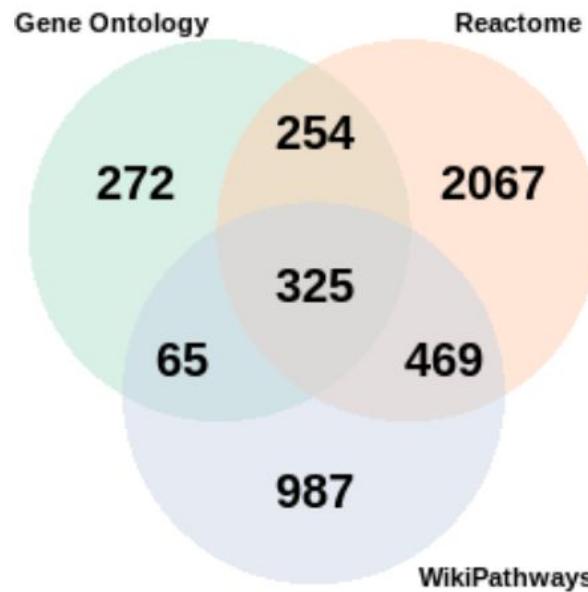
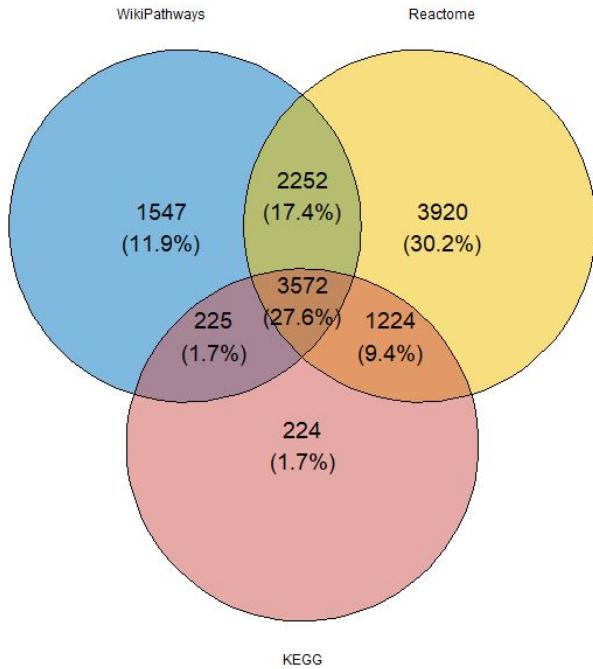


Comparison of the TCA cycle in five metabolic pathway databases. (BiGG, EHMN, HumanCyc, KEGG, Reactome)

Left, Adapted from: Momeni, Zahra, et al. "A Survey on single and multi omics data mining methods in cancer data classification." *Journal of Biomedical Informatics* 107 (2020): 103466. DOI: [10.1016/j.jbi.2020.103466](https://doi.org/10.1016/j.jbi.2020.103466)

Right: Stobbe MD, Houten SM, Jansen GA, van Kampen AH, Moerland PD. Critical assessment of human metabolic pathway databases: a stepping stone for future integration. *BMC Syst Biol.* 2011 Oct 14;5:165. doi: [10.1186/1752-0509-5-165](https://doi.org/10.1186/1752-0509-5-165).

Biological information is scattered among resources

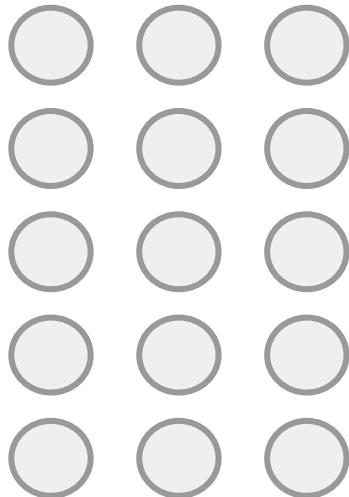


Left: Gene set content overlap analysis between KEGG, Reactome and WikiPathways; data obtained from MSigDb (release 7.4 from April 2021).

Right: Overlapping human lipid metabolism gene terms for three databases (Gene Ontology, Reactome, WikiPathways), based on their HGNC-symbols

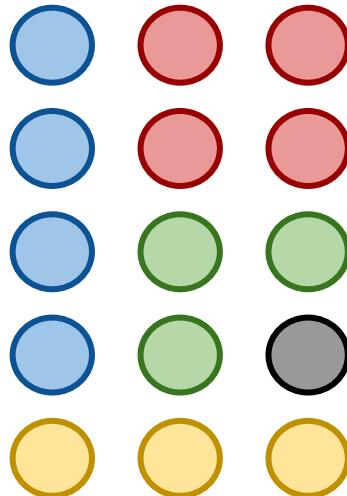
Metabolite/reaction content is harder to compare

Pathway analysis



Quantitative measurements
Isolated data points

Pathway analysis



Comparative statistics

Isolated lists

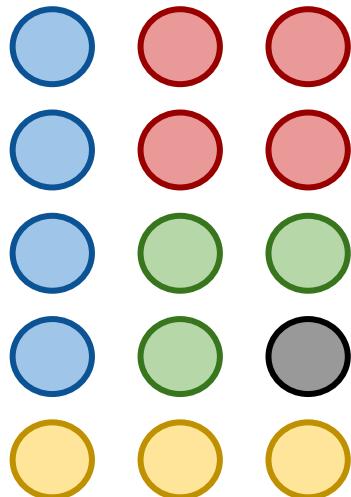
Clustering

Isolated groups

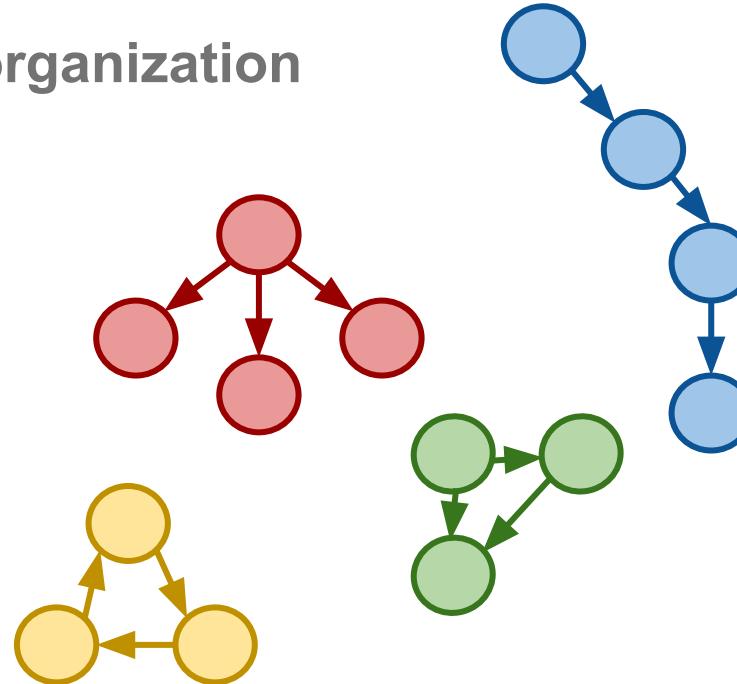
Gene sets

Functional groups

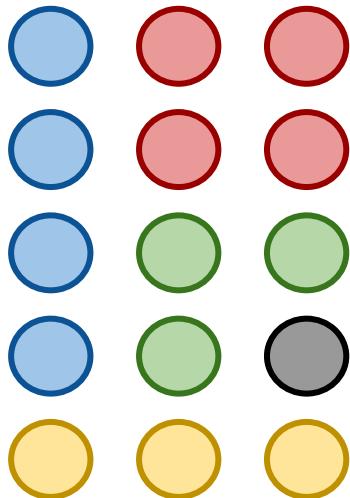
Pathway analysis



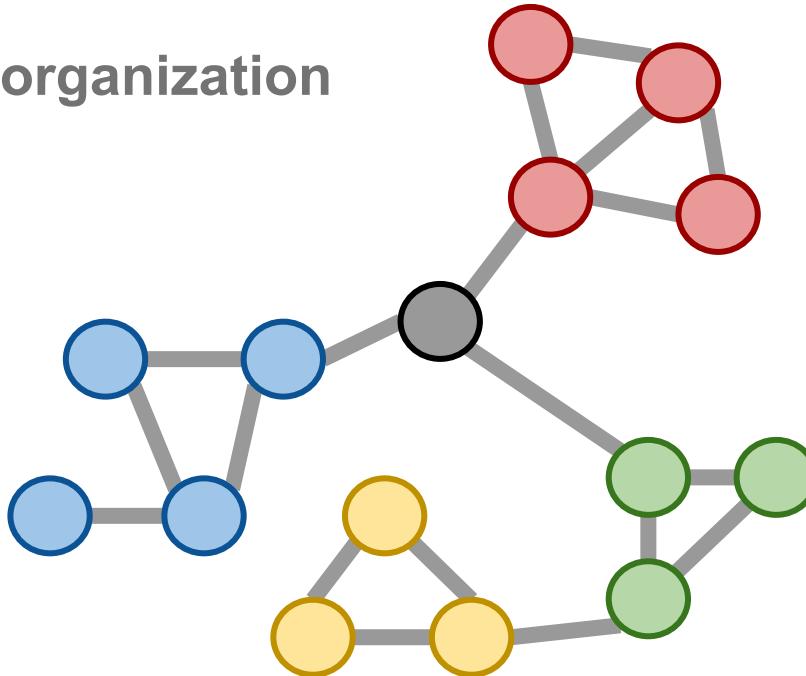
Functional organization
Pathways



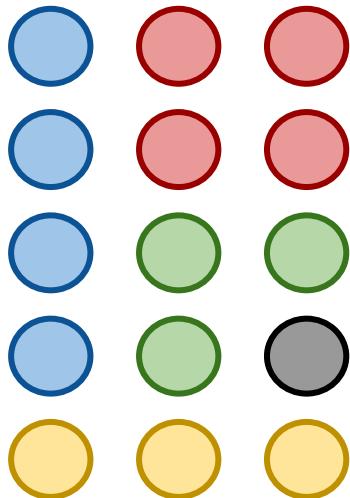
Pathway analysis



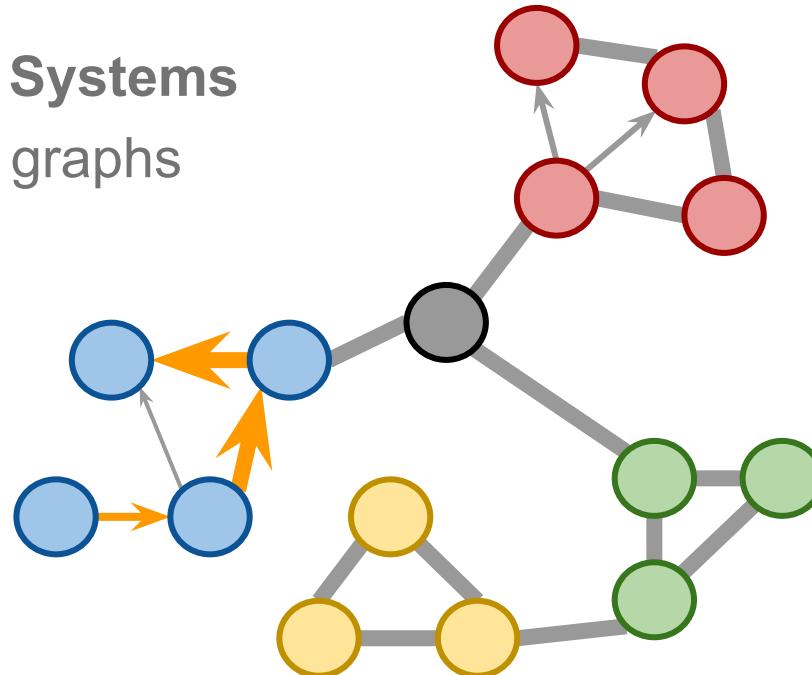
Systems organization
Networks



Pathway analysis

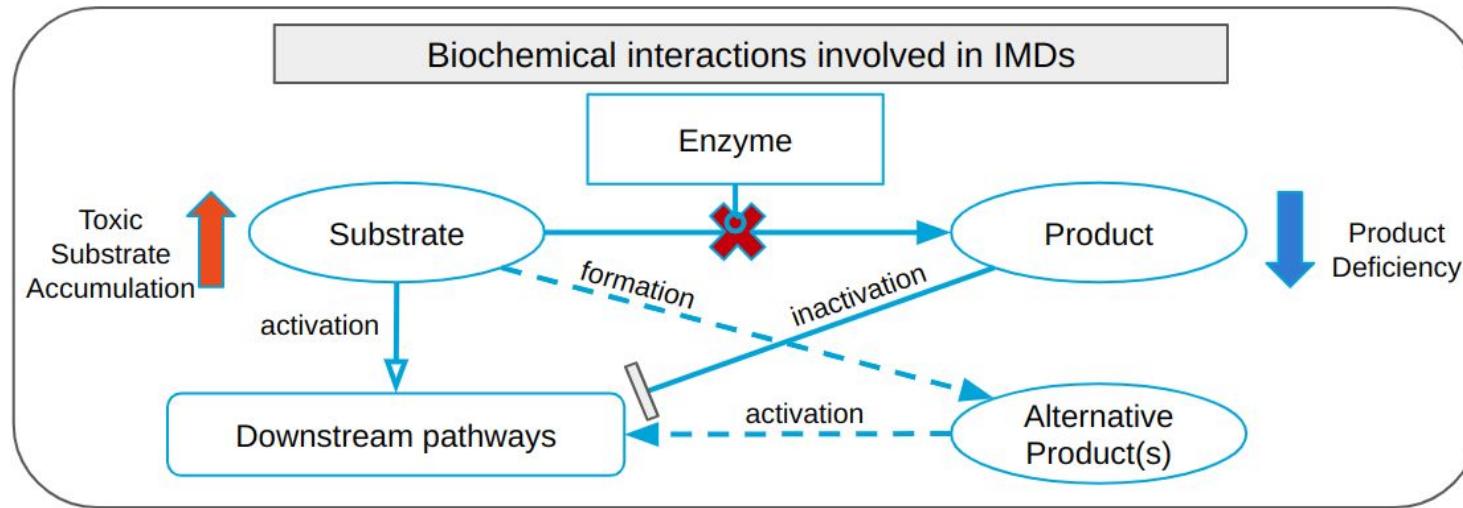


Dynamic Systems
Weighted graphs



Current diagnostics of Inherited Metabolic Disorders (IMDs)

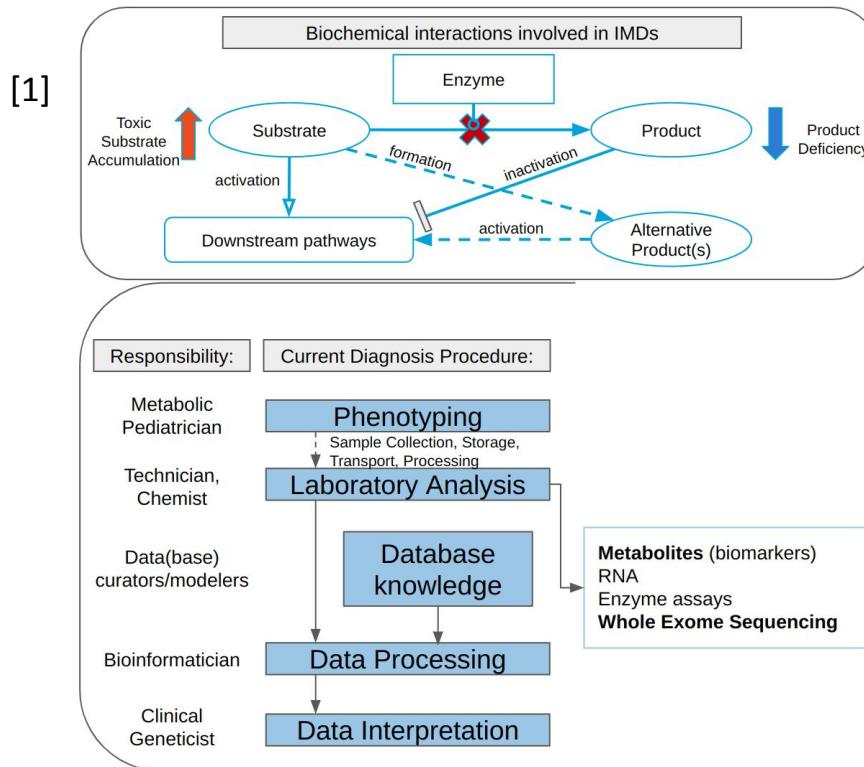
[1]



Overview of biochemical interactions involved in IMDs (top left); the current diagnostic procedure, and challenges in diagnosis using targeted metabolite or WES data for heterogeneous patient populations.

[1] Inspired by Fig. 2 from Lanpher, Nature Reviews Genetics (2006). DOI: [10.1038/nrg1880](https://doi.org/10.1038/nrg1880)

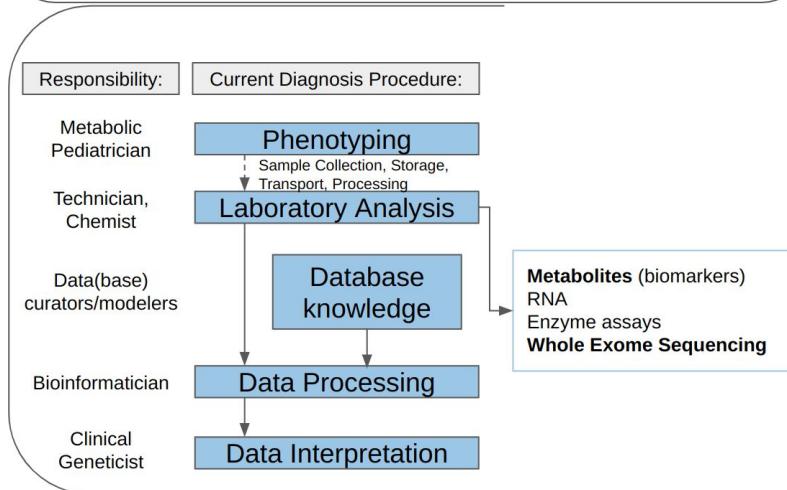
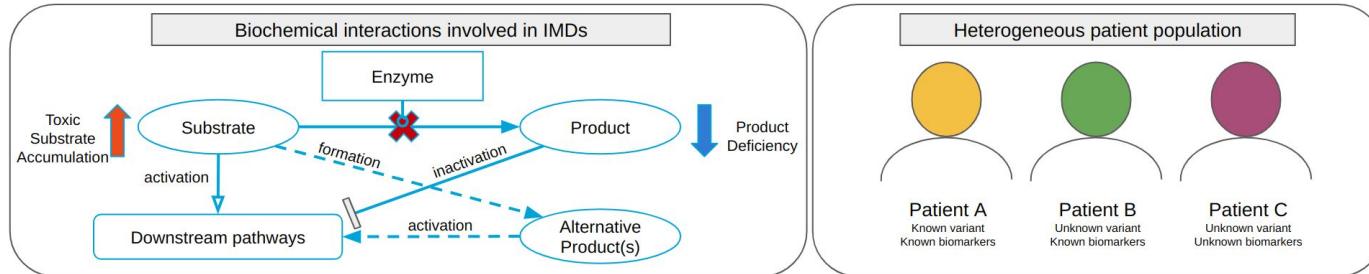
Current diagnostics of Inherited Metabolic Disorders (IMDs)



Overview of biochemical interactions involved in IMDs (top left); the current diagnostic procedure, and challenges in diagnosis using targeted metabolite or WES data for heterogeneous patient populations.

Current diagnostics of Inherited Metabolic Disorders (IMDs)

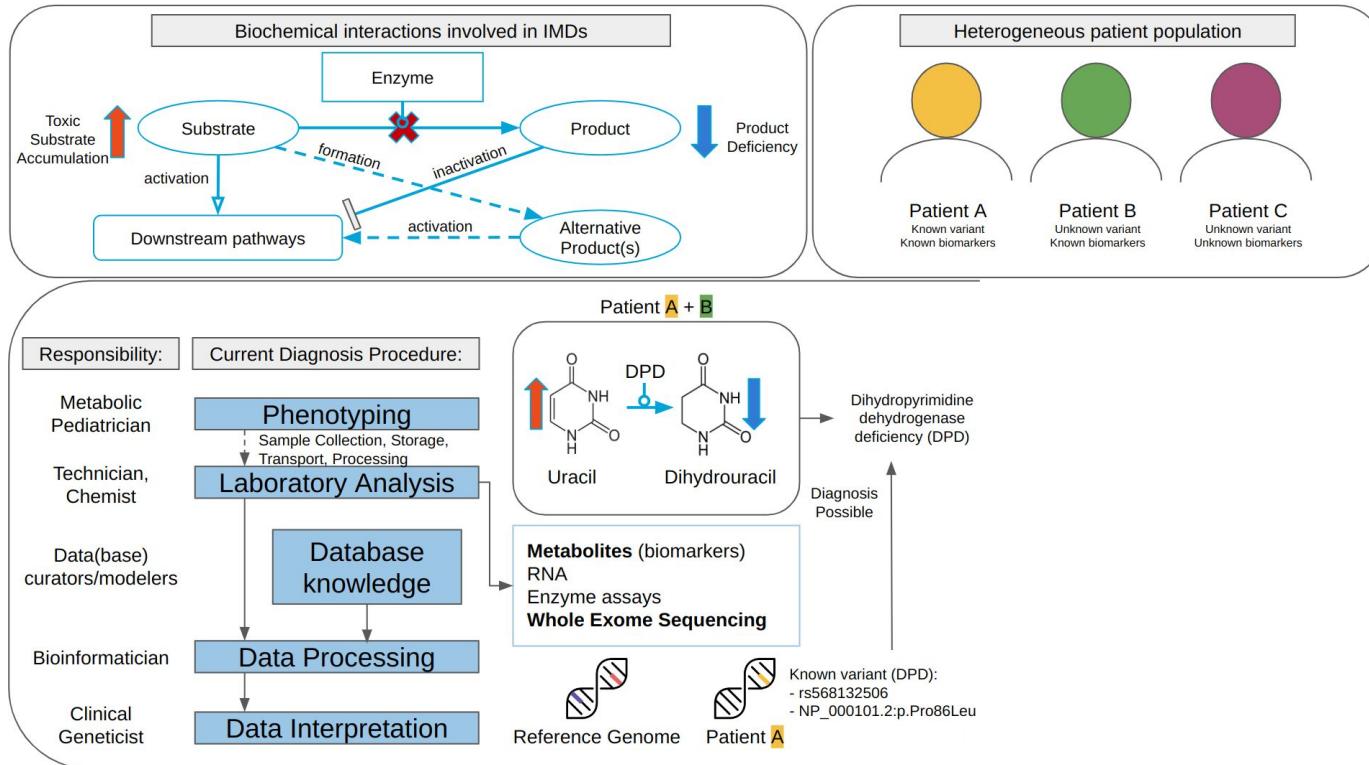
[1]



Overview of biochemical interactions involved in IMDs (top left); the current diagnostic procedure, and challenges in diagnosis using targeted metabolite or WES data for heterogeneous patient populations.

Current diagnostics of Inherited Metabolic Disorders (IMDs)

[1]

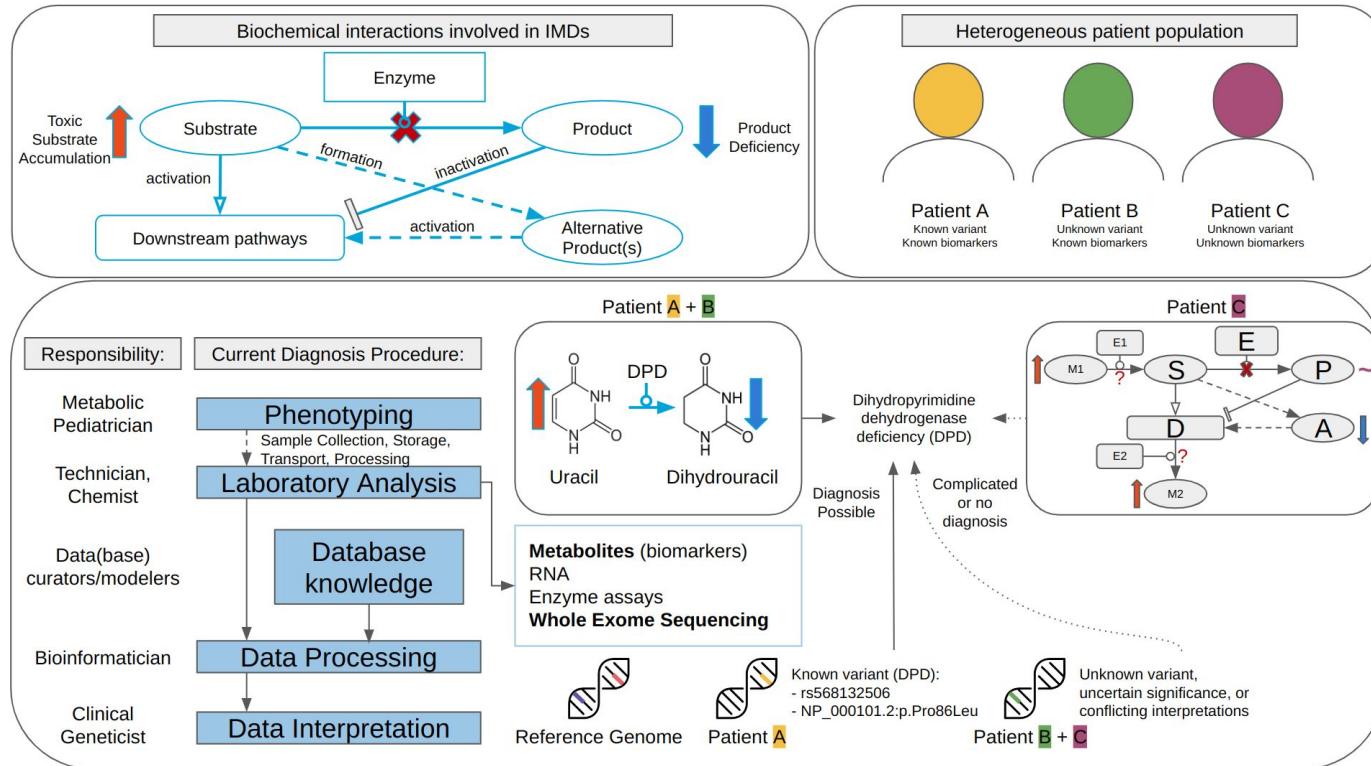


Overview of biochemical interactions involved in IMDs (top left); the current diagnostic procedure, and challenges in diagnosis using targeted metabolite or WES data for heterogeneous patient populations.

[1] Inspired by Fig. 2 from Lanpher, Nature Reviews Genetics (2006). DOI: [10.1038/nrg1880](https://doi.org/10.1038/nrg1880)

Current diagnostics of Inherited Metabolic Disorders (IMDs)

[1]



Overview of biochemical interactions involved in IMDs (top left); the current diagnostic procedure, and challenges in diagnosis using targeted metabolite or WES data for heterogeneous patient populations.

[1] Inspired by Fig. 2 from Lanpher, Nature Reviews Genetics (2006). DOI: [10.1038/nrg1880](https://doi.org/10.1038/nrg1880)

Purposes of Modeling IMDs

Diagnosis challenges:

- Extremely diverse [1], nonspecific clinical presentation [2], genotype–phenotype correlations rarely seen [1]
- Preconception/prenatal screening detects only most common IMDs [2]
- Newborn screening: low prevalence IMDs, but positive predictive values high: 26% to 37% [2] -> >50 FP for 1 TP in USA [3]
- “Snapshot” metabolomic reliably detects null-activity patients, unreliable in partial-activity patients or carriers [1]
- Correlating phenotypes with small changes in metabolic fluxes difficult through genetic tools [1]

[1] Lanpher, *et al.* "Inborn errors of metabolism: the flux from Mendelian to complex diseases." *Nature Reviews Genetics* 7.6 (2006): 449-459. DOI: [10.1038/nrg1880](https://doi.org/10.1038/nrg1880)

[2] Kruszka and Regier. "Inborn errors of metabolism: from preconception to adulthood." *American family physician* 99.1 (2019): 25-32.

[3] Kwon and Farrell. "The magnitude and challenge of false-positive newborn screening test results." *Archives of pediatrics & adolescent medicine* 154.7 (2000): 714-718. DOI: [10.1001/archpedi.154.7.714](https://doi.org/10.1001/archpedi.154.7.714)

Purposes of Modeling IMDs

Diagnosis challenges:

- Extremely diverse [1], nonspecific clinical presentation [2], genotype–phenotype correlations rarely seen [1]
- Preconception/prenatal screening detects only most common IMDs [2]
- Newborn screening: low prevalence IMDs, but positive predictive values high: 26% to 37% [2] -> >50 FP for 1 TP in USA [3]
- “Snapshot” metabolomic reliably detects null-activity patients, unreliable in partial-activity patients or carriers [1]
- Correlating phenotypes with small changes in metabolic fluxes difficult through genetic tools [1]

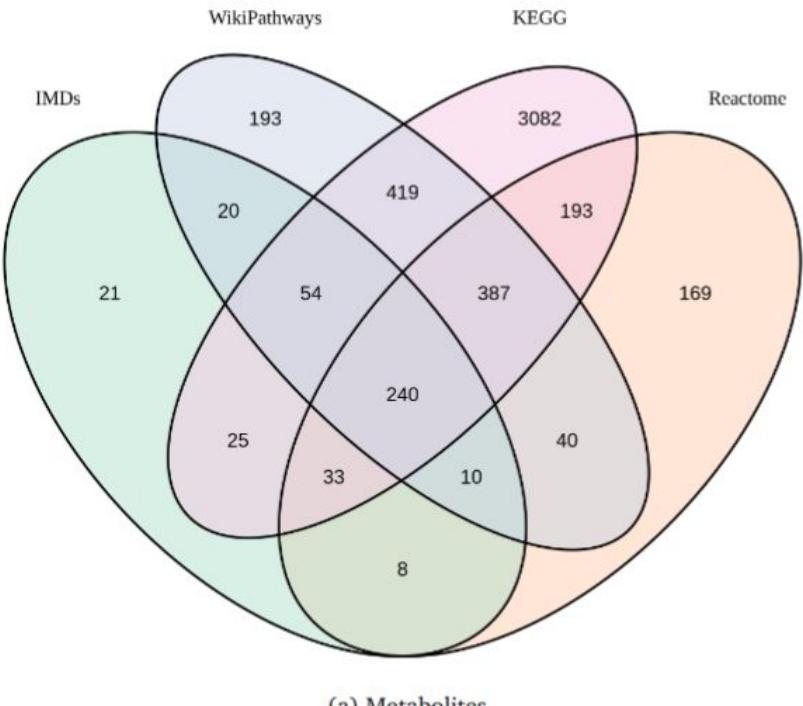
Data driven approaches:

- Previously regarded as Mendelian traits; now examples of complex gene–environment/-nutrient interactions -> complex diseases [1]
- Understanding the full metabolome to understand complex disease pathogenesis and susceptibility [1]
- Metabolite flux integrate environmental, genetic, biochemical factors with phenotype; helps diagnosis and therapy development [1]
- Effective therapy requires metabolite flux alterations [1]

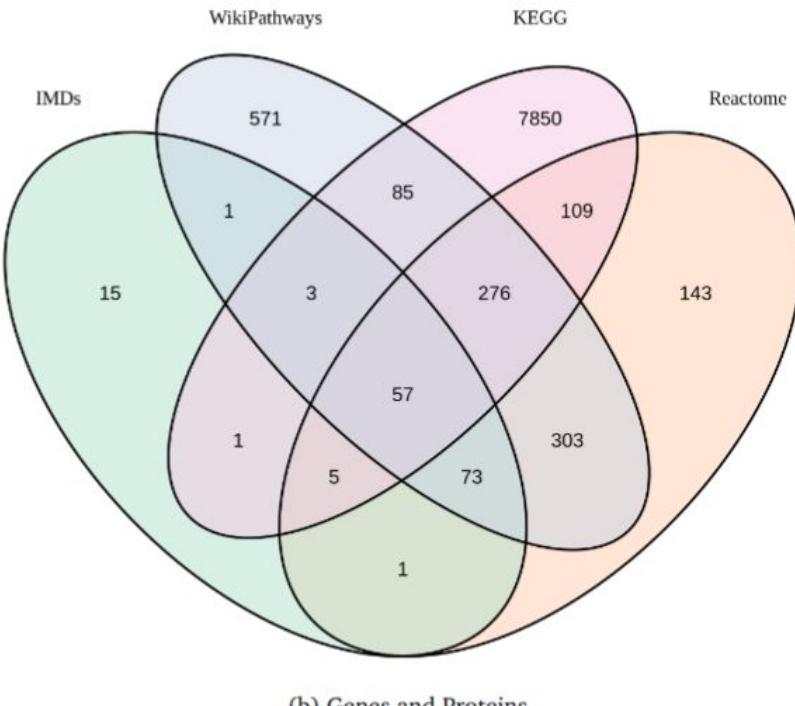
[1] Lanpher, et al. "Inborn errors of metabolism: the flux from Mendelian to complex diseases." *Nature Reviews Genetics* 7.6 (2006): 449-459. DOI: [10.1038/nrg1880](https://doi.org/10.1038/nrg1880)

[2] Kruszka and Regier. "Inborn errors of metabolism: from preconception to adulthood." *American family physician* 99.1 (2019): 25-32.

[3] Kwon and Farrell. "The magnitude and challenge of false-positive newborn screening test results." *Archives of pediatrics & adolescent medicine* 154.7 (2000): 714-718. DOI: [10.1001/archpedi.154.7.714](https://doi.org/10.1001/archpedi.154.7.714)



(a) Metabolites



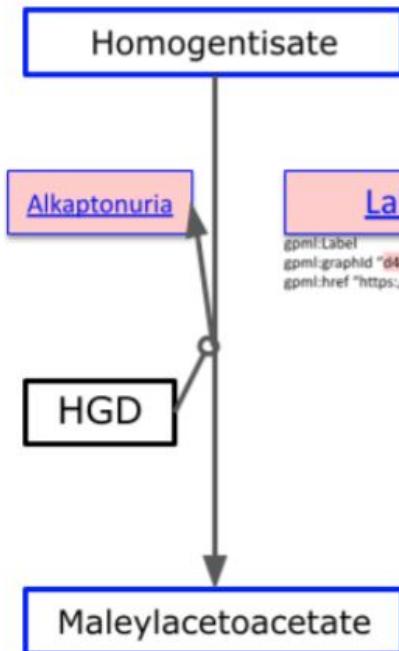
(b) Genes and Proteins

Fig. 4 Overview of metabolite and gene/protein pathway content from three pathway databases (KEGG, Reactome, WikiPathways) compared to the content from the IMD models. Metabolites were unified to the KEGG Compound ID, genes and proteins to the Entrez (NCBI) gene ID using BridgeDb.

IMDs through Semantic web approach (RDF)

- Requires clear PW modeling guidelines
- How to connect Disease info to PWMs?
- Which biology cannot be captured now?

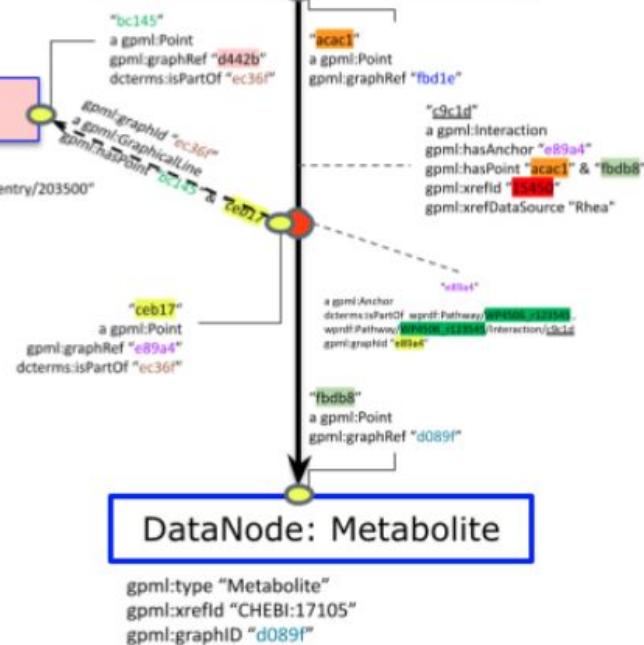
GPML (2013)



GPML-RDF

gpmi:type "Metabolite"
gpmi:xrefId "CHEBI:16169"
gpmi:graphID "fb1de"

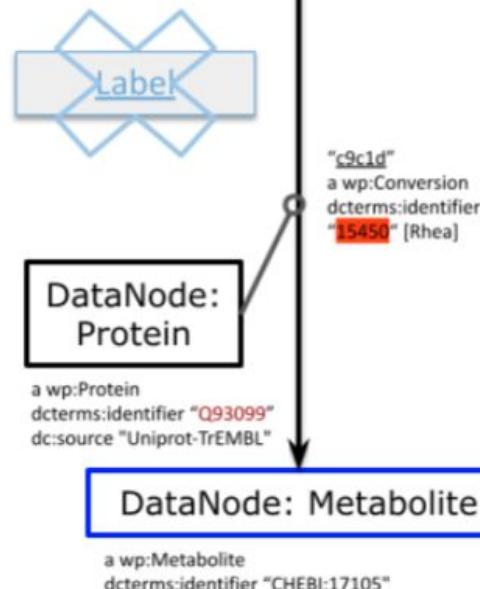
DataNode: Metabolite



WP-RDF

a wp:Metabolite
dcterms:identifier "CHEBI:16169"
dcterms:isPartOf
wprdf:Pathway/WP4506/c12354d/WP/Interaction/c9c1d

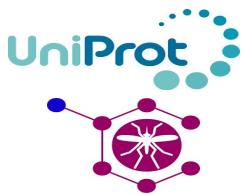
DataNode: Metabolite



Example disease interaction: [WP4506](#) Tyrosine metabolism and related disorders

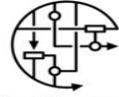
Fig. 3 Pathway model data captured in GPML(2013), GPML-RDF, and WP-RDF schema, in the case of one substrate (homogentisate) being converted to one product (maleyl acetoacetate) by one protein (HGD), which is linked to the IMD alkaptonuria.

Kinetic Data is also Scattered among Resources

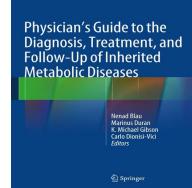


Maastricht University

LINK



WIKIPATHWAYS
Pathways for the People



RESEARCH PAPER | Open Access | CC BY

Is systems pharmacology ready to impact upon therapy development? A study on the cholesterol biosynthesis pathway

Helen E Benson, Steven Watterson, Joanna L Sharman, Chido P Mpamhangwa, Andrew Parton, Christopher Southan, Anthony J Harmar, Peter Ghazal

First published: 14 September 2017 | <https://doi.org/10.1111/bph.14037> | Citations: 4

Maastricht University find full text

Current address: TB Section, Respiratory Disease Department, National Infection Service, Public Health England, 61 Colindale Avenue, London NW9 5EQ, UK.

SECTIONS

PDF TOOLS SHARE

Abstract

Background and Purpose

An ever-growing wealth of information on current drugs and their pharmacological effects is available from online databases. As our understanding of systems biology increases, we have the opportunity to predict, model and quantify how drug combinations can be introduced that outperform conventional single-drug therapies. Here, we explore the feasibility of such systems pharmacology approaches with an analysis of the mevalonate branch of the cholesterol biosynthesis pathway.

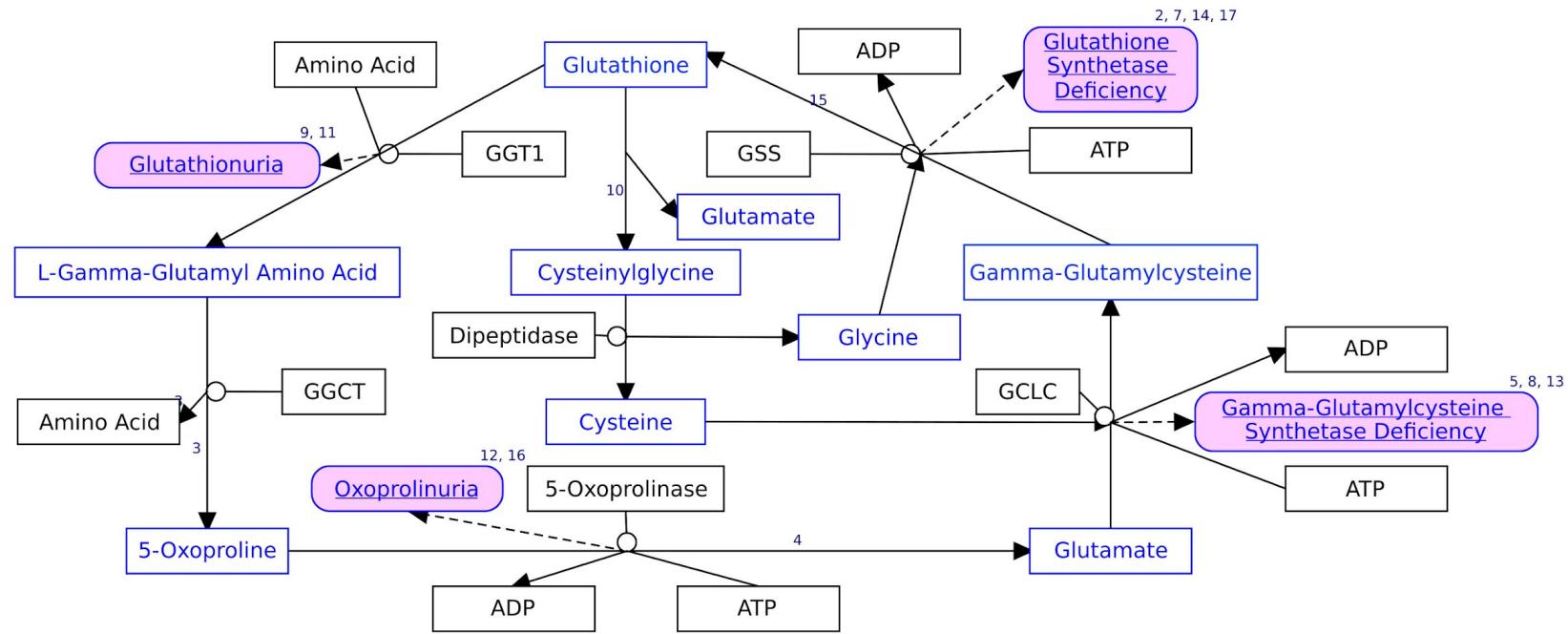


Figure 1: Gamma-Glutamyl Cycle for the biosynthesis and degradation of glutathione, including diseases (*Homo sapiens*). <https://www.wikipathways.org/instance/WP4518>

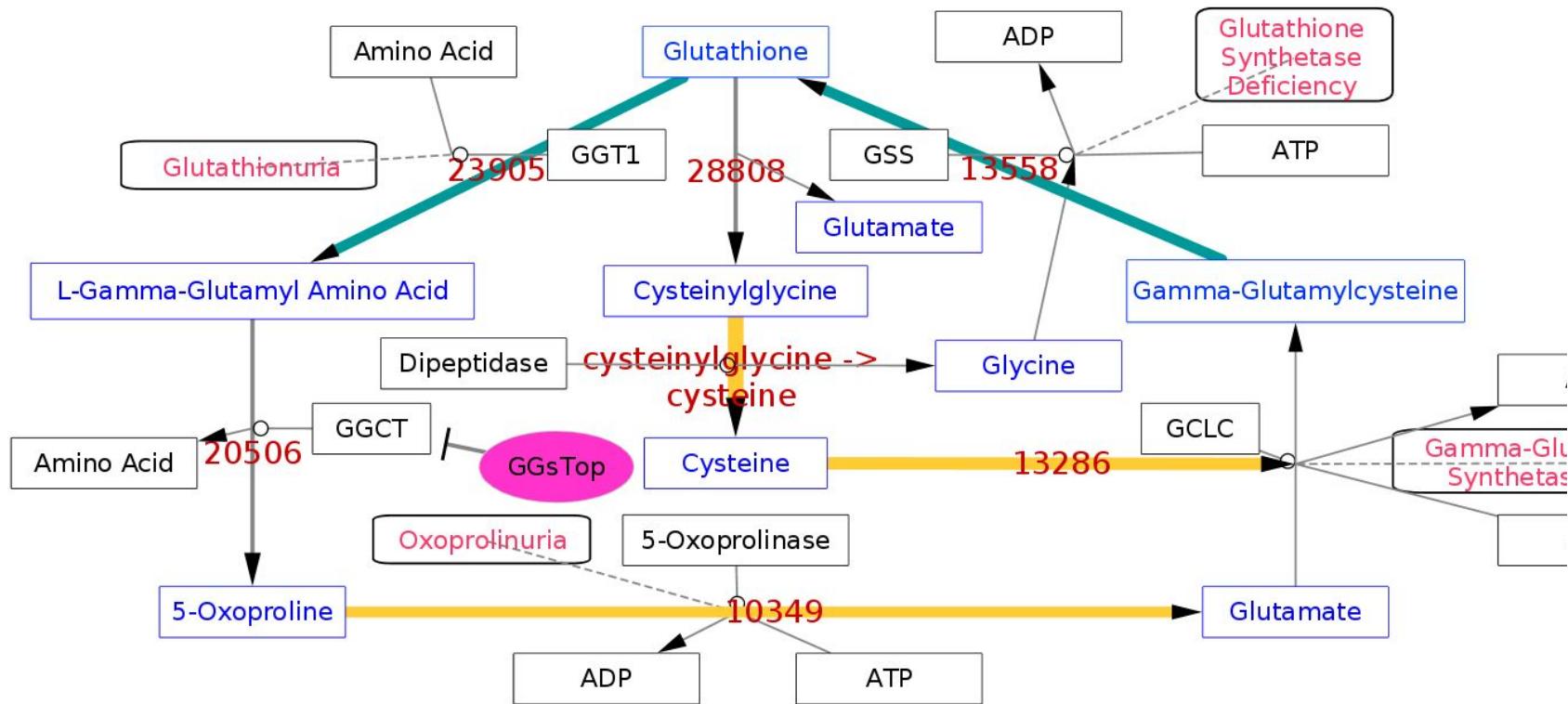
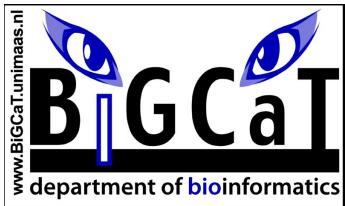


Figure 5: Combining pathways through Rhea identifiers (red) with Km values (edge thickness) for two species: *Homo sapiens* (green) and *Rattus norvegicus* (yellow) and inhibitor (pink).

Acknowledgements, questions, discussion

- Jonathan Mélius
- Georg Summer
- Ammar Ammar
- Tooba Abbassi-Daloii
- WikiPathways: team and curators
- Reactome
- Lipid Maps
- Metabolights
- Elixir



Additional Data:

Table 1 Overview of side metabolites for the DSMN.

		Biological role			
Identifier	Name	Identifier	Name	Identifier	Name
Q5203615	O ₂	Q80863,	ATP	Q307434	S-adenosyl-L-homocysteine
		Q27113900	ATP ⁴⁻		
Q506710	H ⁺	Q185253,	ADP,	Q201312	S-adenosyl-L-methioninate
		Q27225748	ADP ³⁻		
Q3154110	Na ⁺	Q318369	AMP	Q407635	Coenzyme-A
Q283	H ₂ O	Q422582	GDP	Q715317	Acetyl coenzyme a
Q171877	H ₂ O ₂	Q392227	GTP	Spurious identifiers	
Q1997	CO ₂	Q26987754	NADP ⁺	Identifier	Name
Q177811,	PO ₄ ³⁻ ,	Q26841327	NADPH	Q7430	DNA
Q27104508	HPO ₄ ⁻			Q11053	RNA
Q411092	Pyrophosphoric acid	Q26987253,	NAD ⁺ ,	Q172290	Sulfate ion
		Q28529711	NAD ⁻	Q427071Q2225	Hydroxyl radical electron
Q190901	ammonium cation NH ₄ ⁺	Q26987453,	NADH,	Q428946	Iron(II)
Q4087	Ammonia NH ₃	Q27125072	NADH ²⁻	Q3233795	Iron(III)
		Q27102690	FADH ₂	Q24301658	L-amino acid

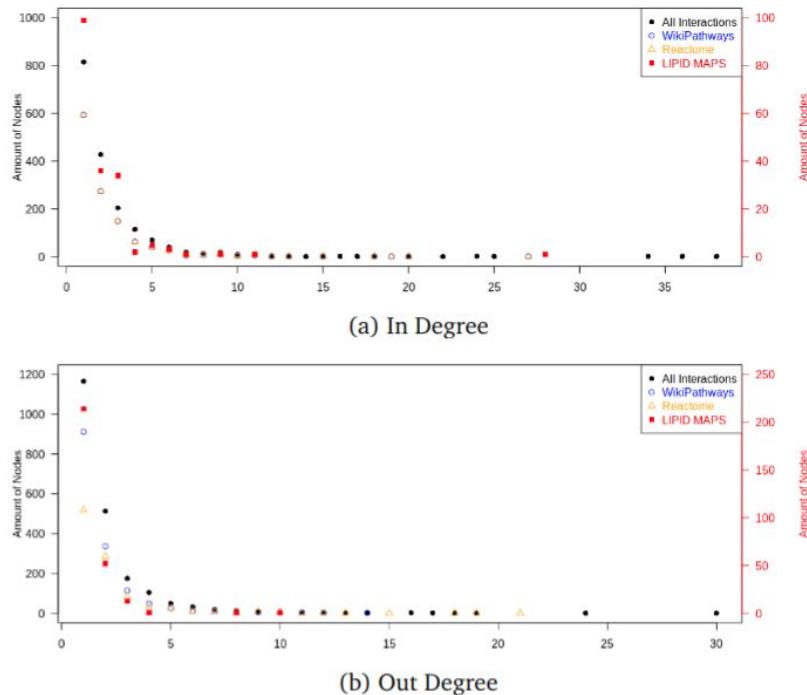


Fig. 2 Network topology inspection by degree. Visualization of In (a) and Out (b) degrees of the total DSMN graph, showing metabolic reactions belonging to the four different collections in the graph database: All, WikiPathways, Reactome, and LIPID MAPS. The latter was the smallest collection and is therefore depicted on an individual scale (right vertical axis).