Multiomic integration and analysis through graphs

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Overview

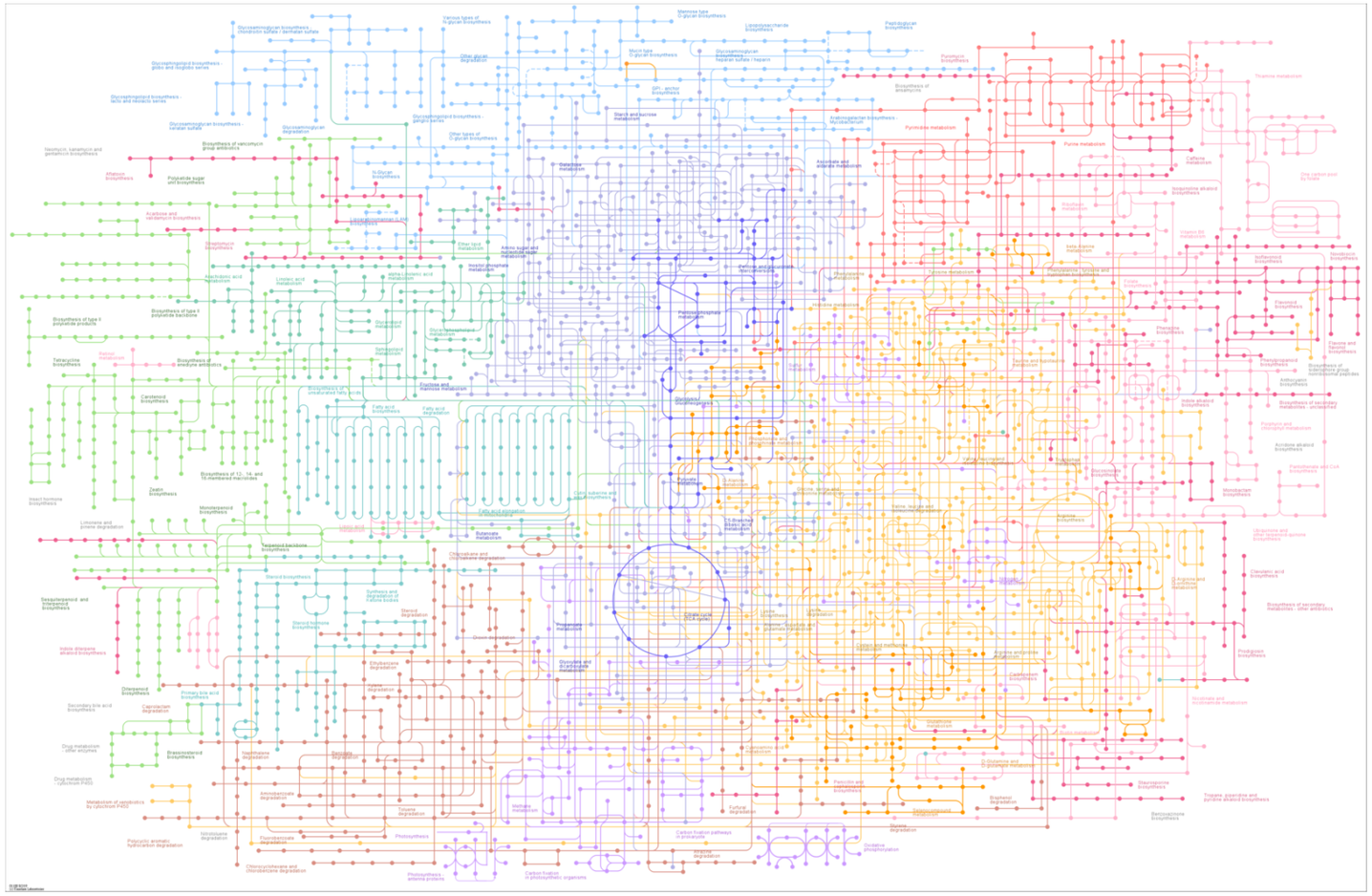
Overall objective

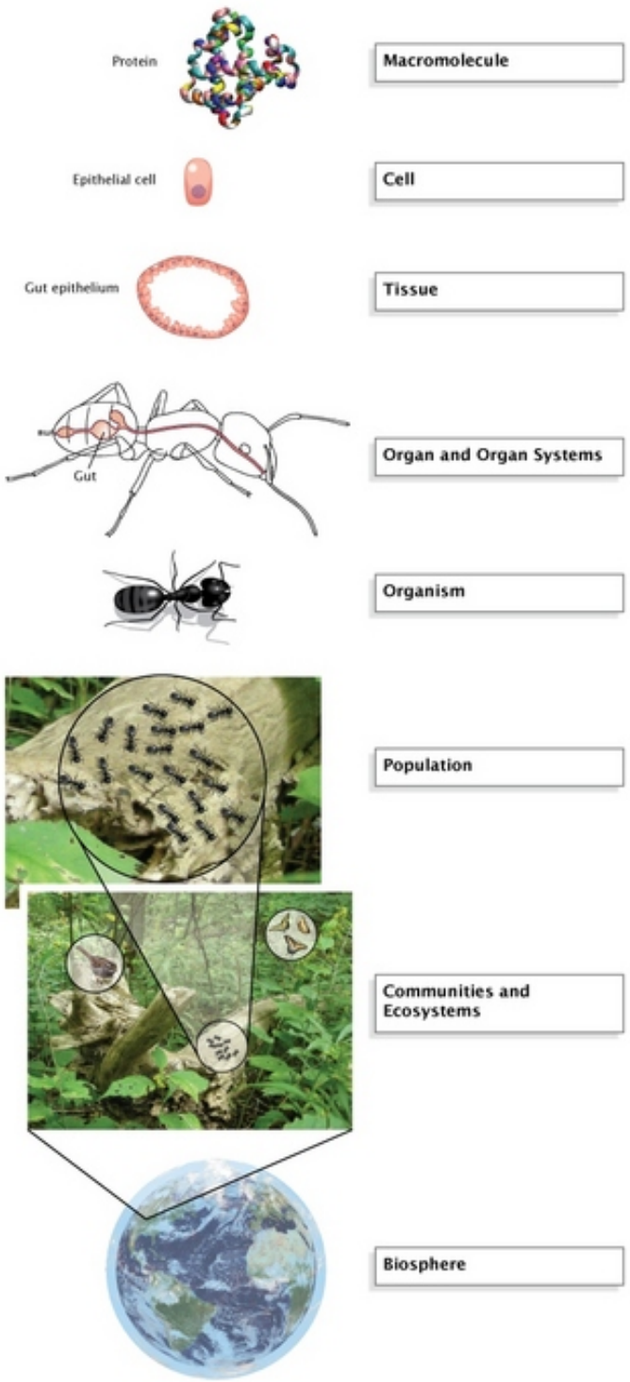
1. Introduction to network analysis
2. Terminology
3. Network construction
4. Key network properties
5. Community analysis
6. Workshop

This lecture is a condensed version of that taught at the Omics Integration and Systems Biology course

Introduction

Biological complexity under attack



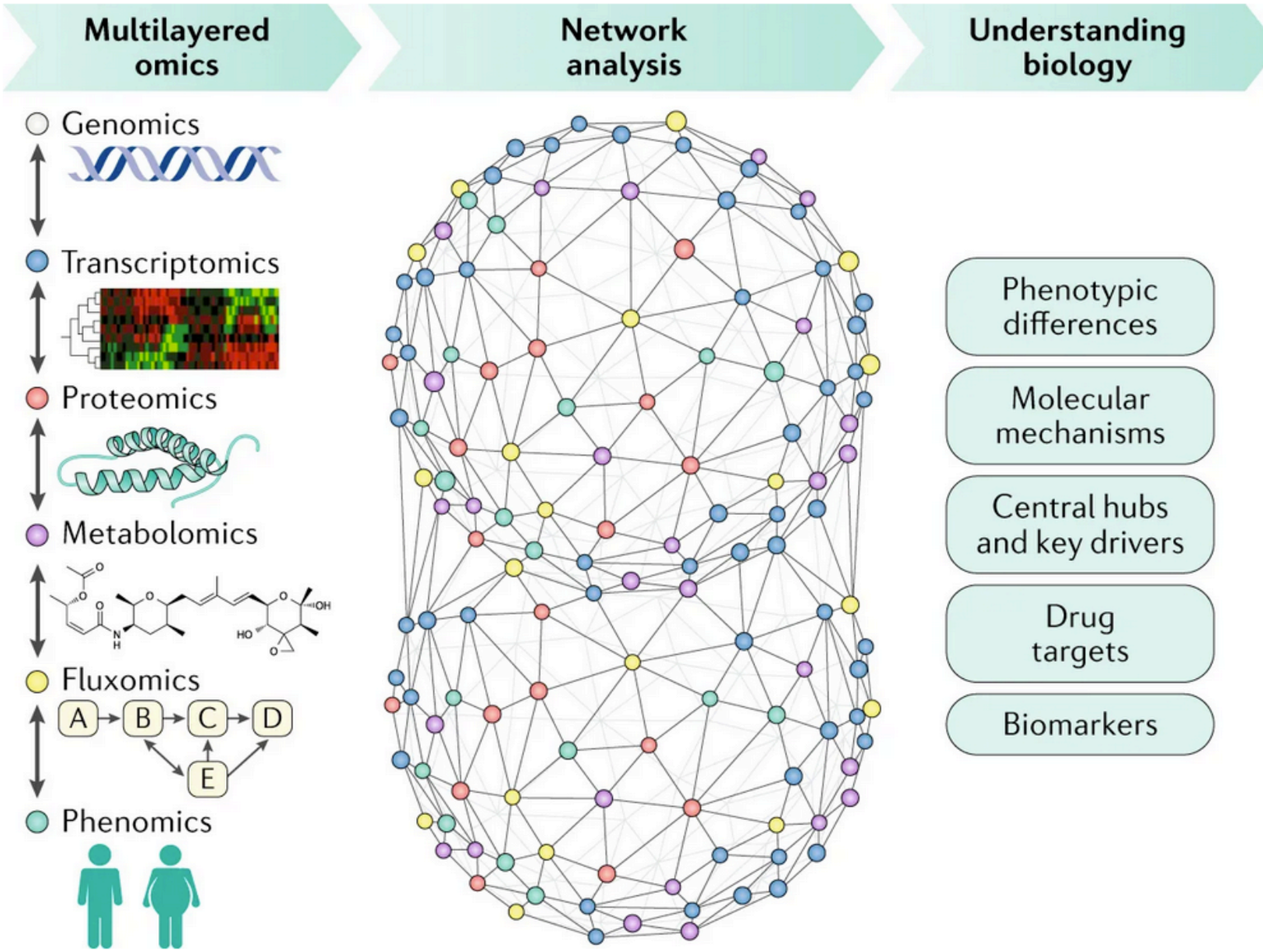


Lobo 2008
genome.jp/kegg/

How to tackle biological complexity?

Integrative approaches, and global patterns

- Feature association
- Modeling (e.g. GEMs)
- Network analysis

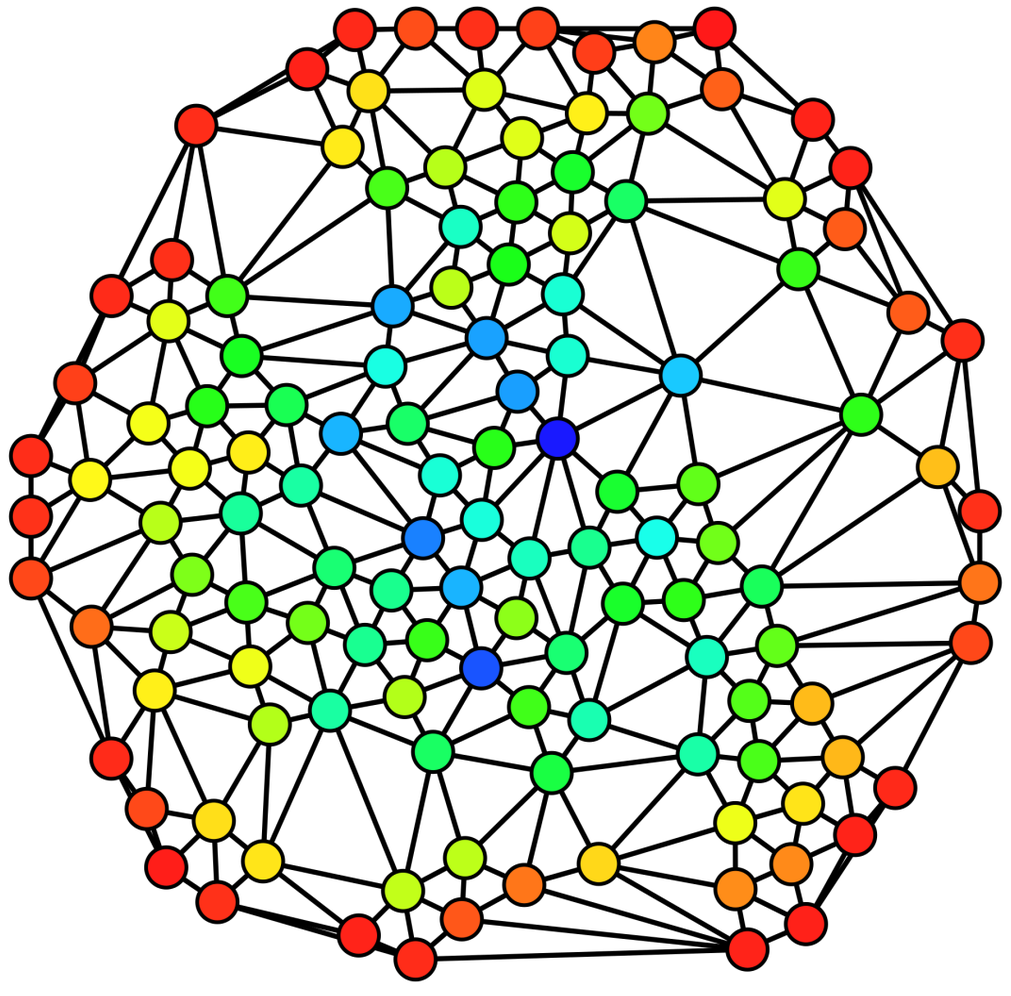


What are networks?

Networks are representations of complex systems

Give us insight not easily achieved by other approaches:

- Comprehensive
- Coordinated (e.g. from diff. exp)
- Single- vs multiomic responses



What are biological networks?

?

What are biological networks?

Protein - Protein interaction (PPI) networks

Transcription-factor regulatory networks

Gene - gene co-expression networks

Signal transduction networks

Transcription-Factor Regulatory networks

Drug-disease association networks

Aim

Functional / mechanistic characterisations

What are biological networks?

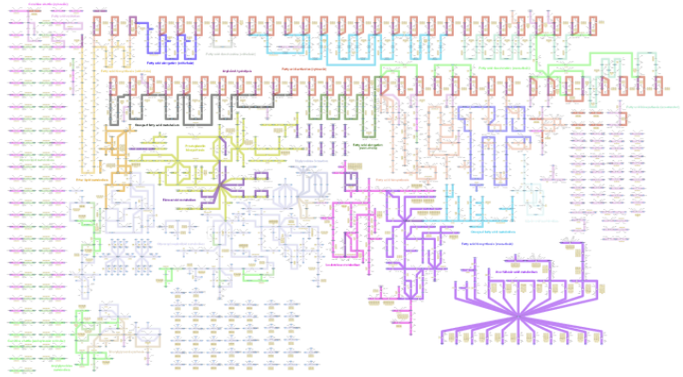
Metabolite - Enzyme - Signal - Genes (GEMs)

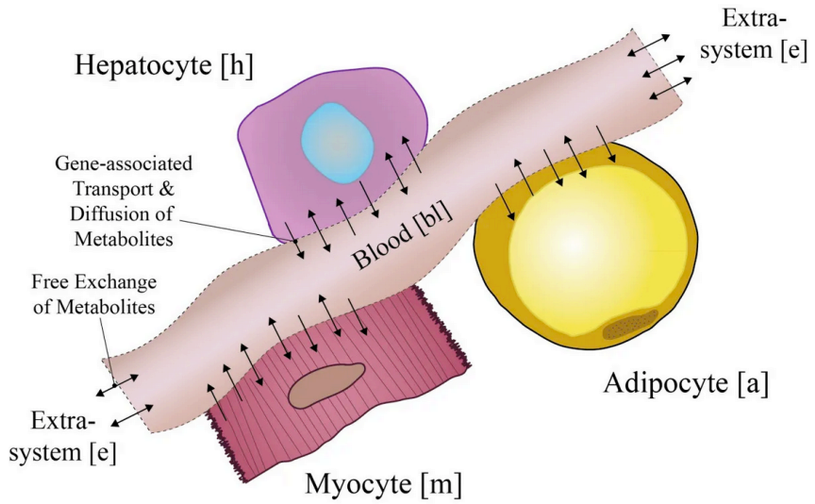
Multi-tissue networks

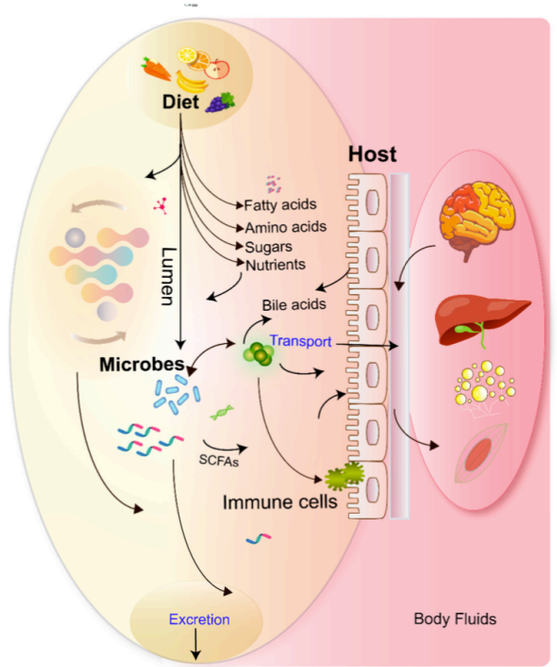
Multi-species networks

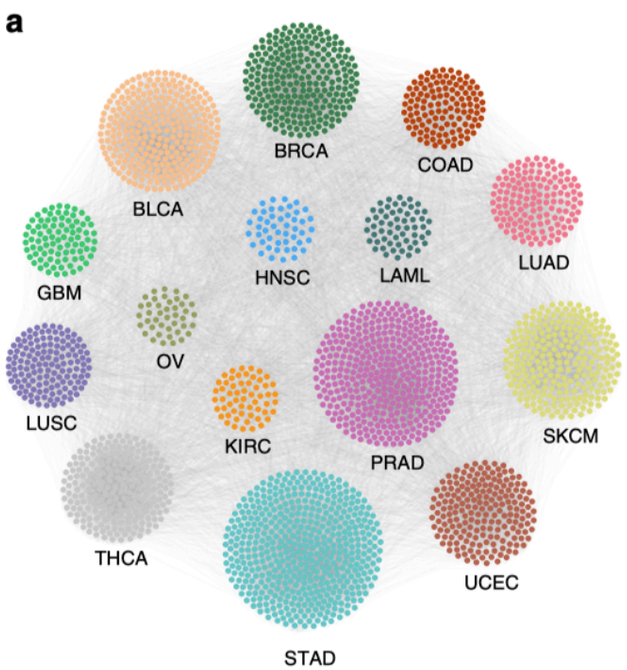
Cancer/disease networks

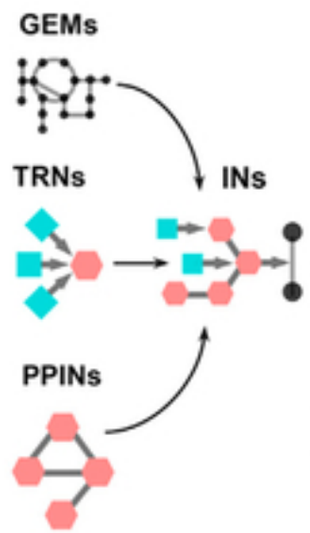
Integrated networks











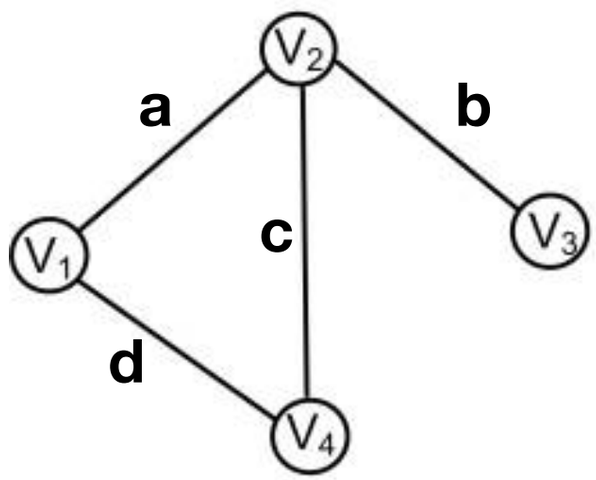
Terminology and initial properties in graph analysis

Graphs, nodes, edges

Graph *G* consists of a set of **nodes** (V) interconnected by **edges** (E)

Nodes sometimes called **vertices**

Two connected nodes: **neighbours**



Simple vs multigraphs

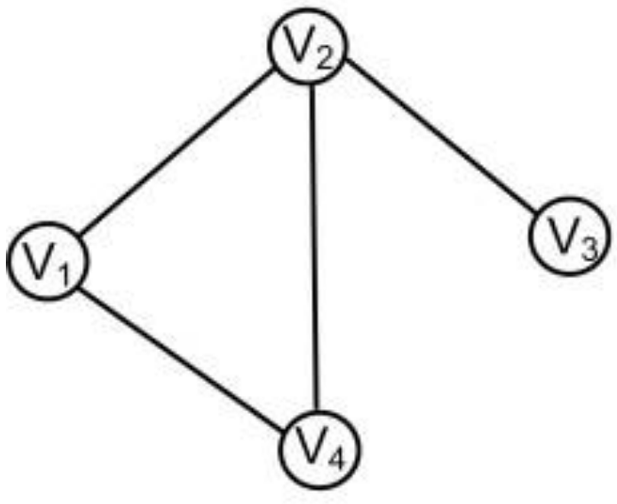
Multigraphs contain parallel edges

Multi-edged connections indicate different properties

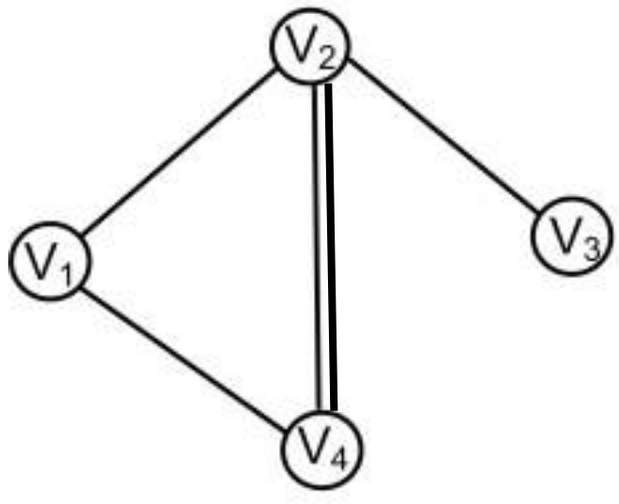
Example: PPI

- Experimental evidence for interaction
- Co-expression

Simple



Multigraphs

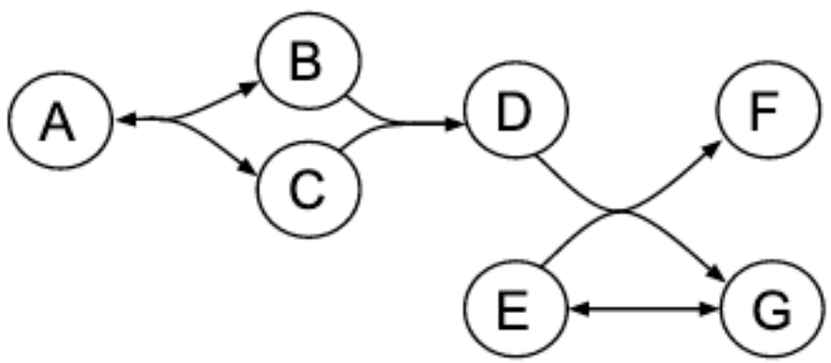


Hypergraphs

Hypergraphs contain edges that connect any number of nodes

Reaction 1: $A \rightarrow B + C$
Reaction 2: $B + C \rightarrow D$
Reaction 3: $D + E \rightarrow F + G$
Reaction 4: $E \rightarrow G$
Reaction 5: $B + C \rightarrow A$
Reaction 6: $G \rightarrow E$

(a) Reaction network

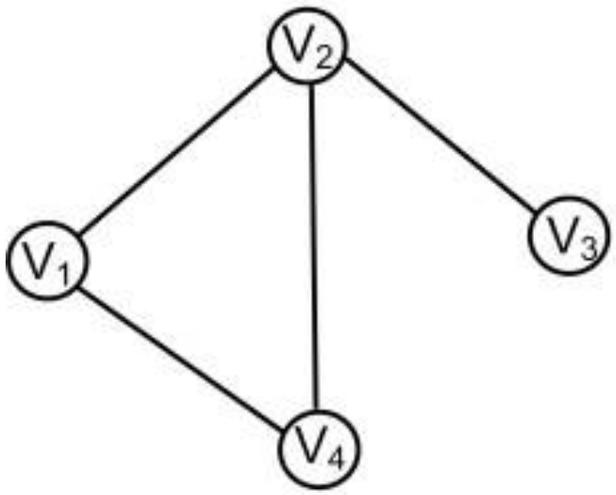


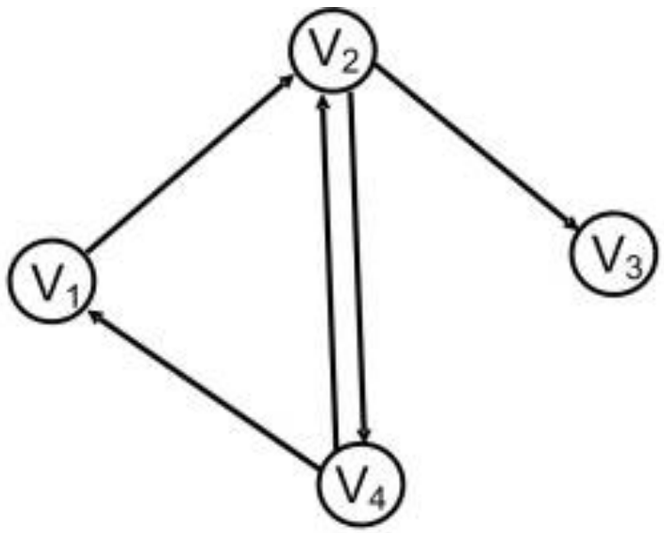
Directed vs undirected graphs

Examples:

- **Undirected graphs:** co-expression networks

- **Directed graphs:** metabolic networks



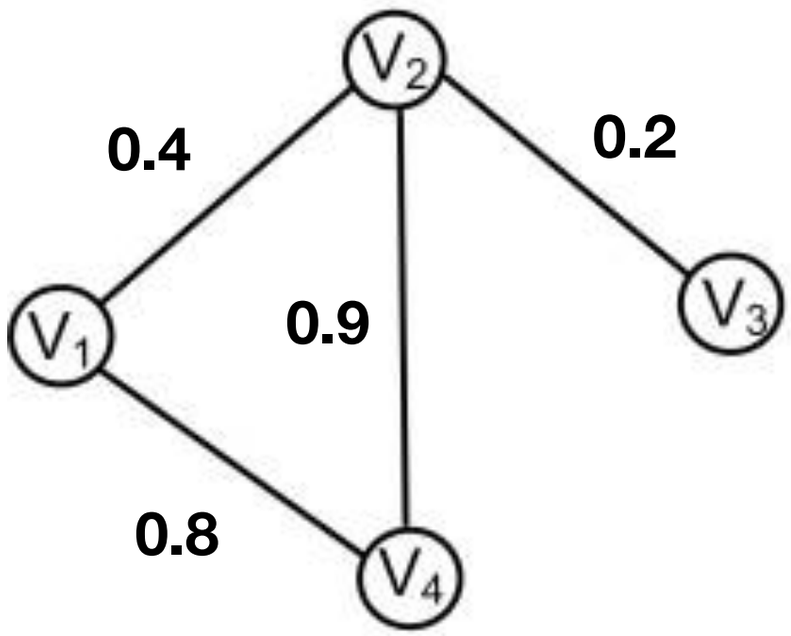


Weighted vs unweighted graphs

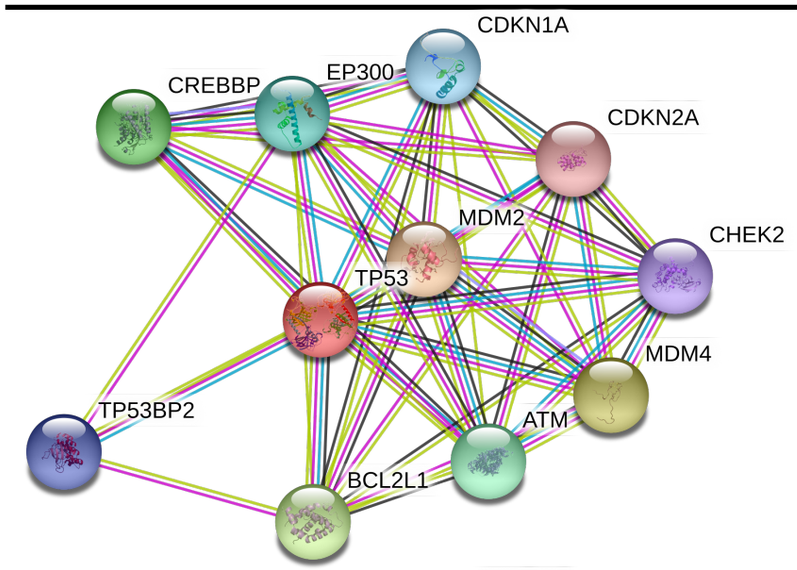
Weighted edges associate a value to an interaction between two nodes. E.g. weights ~ confidence in the interaction.

Negative weights?

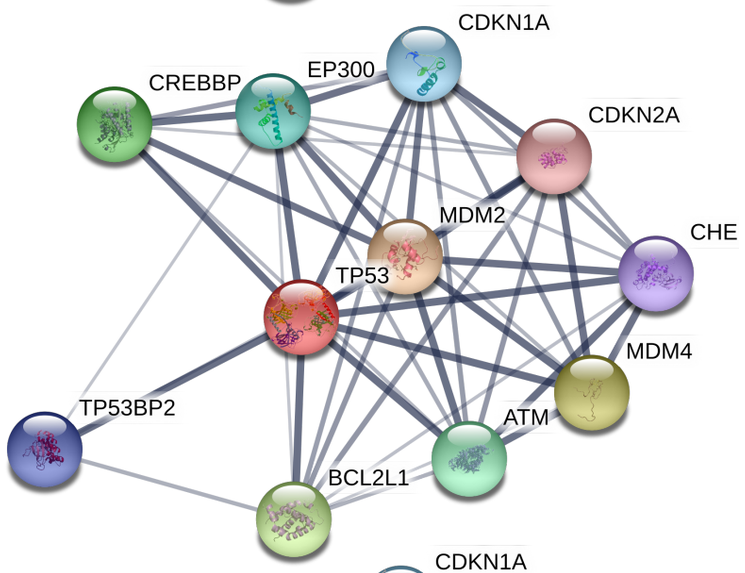
E.g. weighted co-expression networks



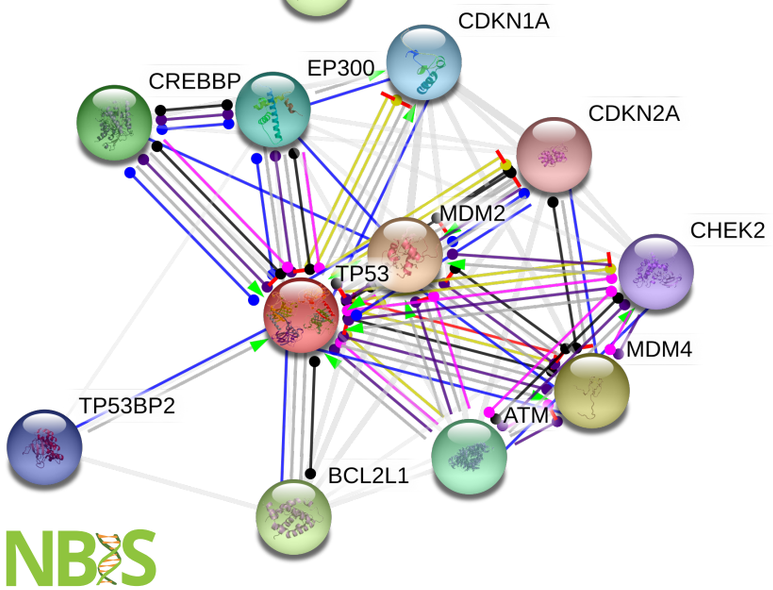
STRING-db.org: TP53



Multi-edged



Weighted multi-edged

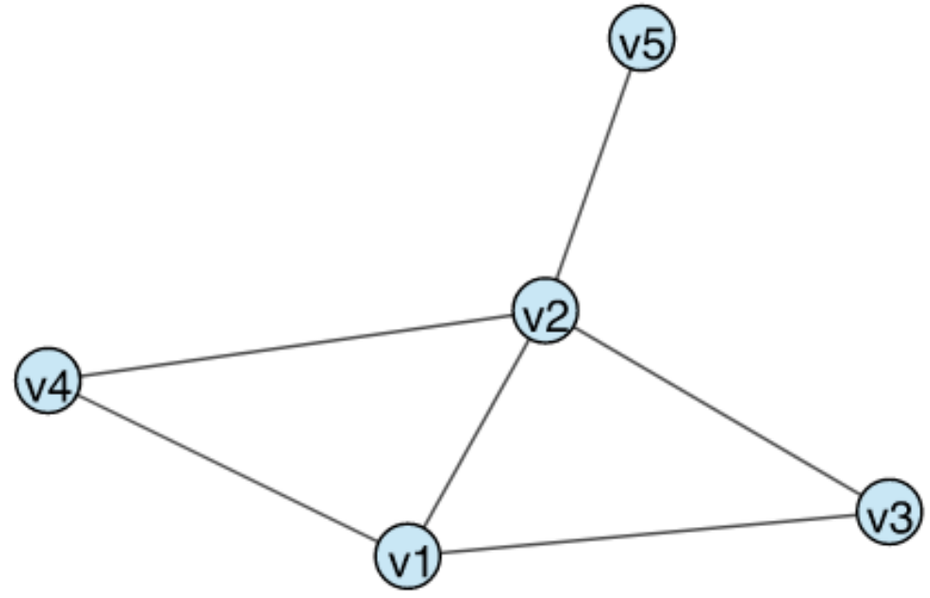


Multi-edged directed

Adjacency matrix (undirected graphs)

Vertex association (undirected network)

n1	n2
v1	v2
v1	v4
v2	v4
v2	v3
v2	v5
v1	v3



Adjacency matrix is symmetric

	v1	v2	v3	v4	v5
v1	0	1	1	1	0
v2	1	0	1	1	1
v3	1	1	0	0	0
v4	1	1	0	0	0
v5	0	1	0	0	0

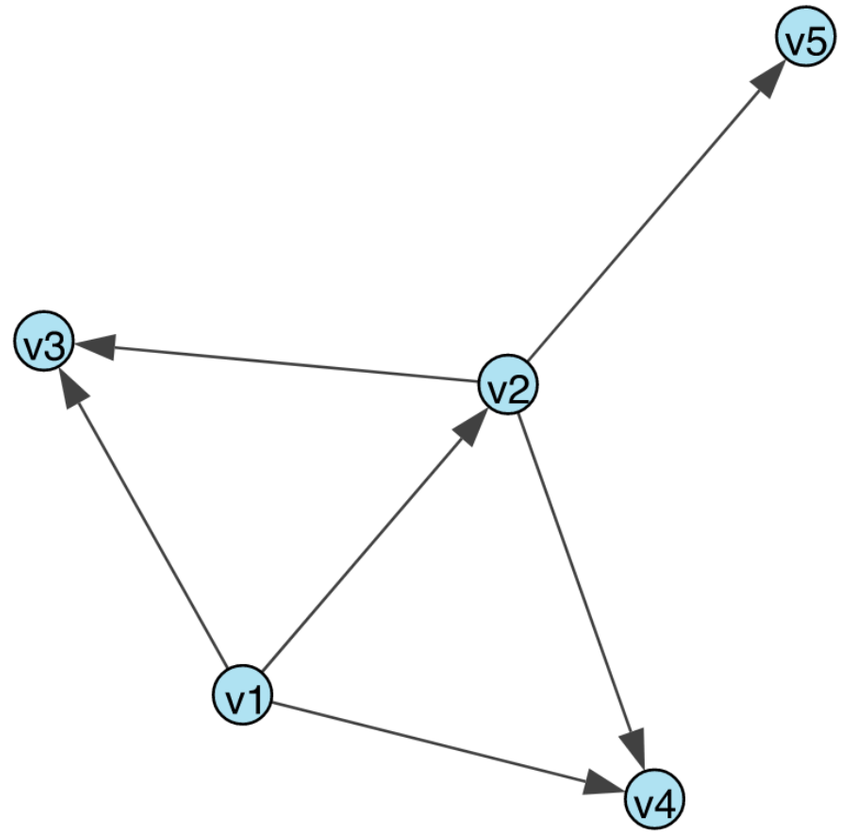
Upper triangular

Diagonal

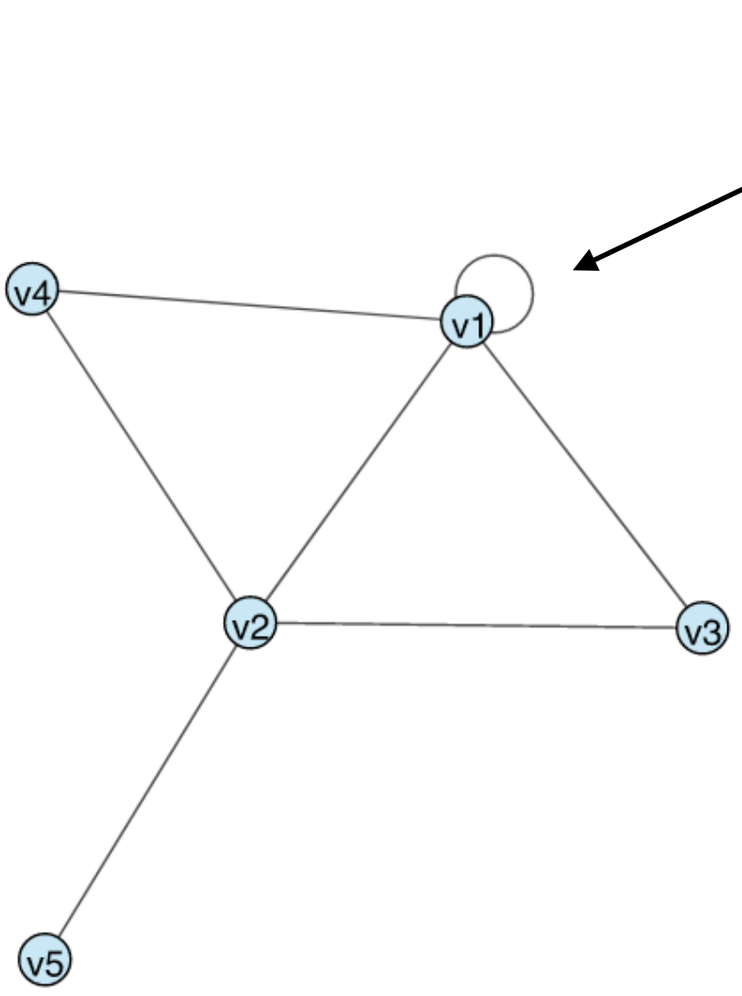
Lower triangular

Adjacency matrix (directed graphs)

Source \ Target	v1	v2	v3	v4	v5
v1	0	1	1	1	0
v2	0	0	1	1	1
v3	0	0	0	0	0
v4	0	0	0	0	0
v5	0	0	0	0	0



Graphs may contain self-loops



Loop

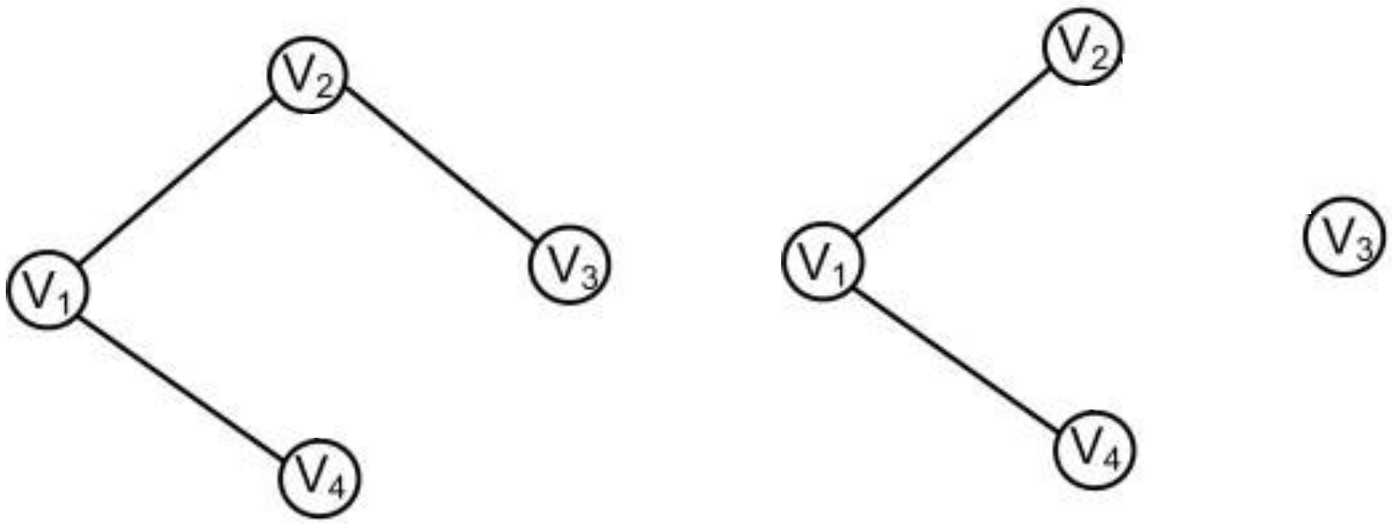
	v1	v2	v3	v4	v5
v1	**1**	1	1	1	0
v2	1	0	1	1	1
v3	1	1	0	0	0
v4	1	1	0	0	0
v5	0	1	0	0	0

Examples of **self-loops** are auto-regulatory mechanisms (feed-back loops)

Connected vs disconnected networks

Connected network: there is at least 1 path connecting all nodes in a network

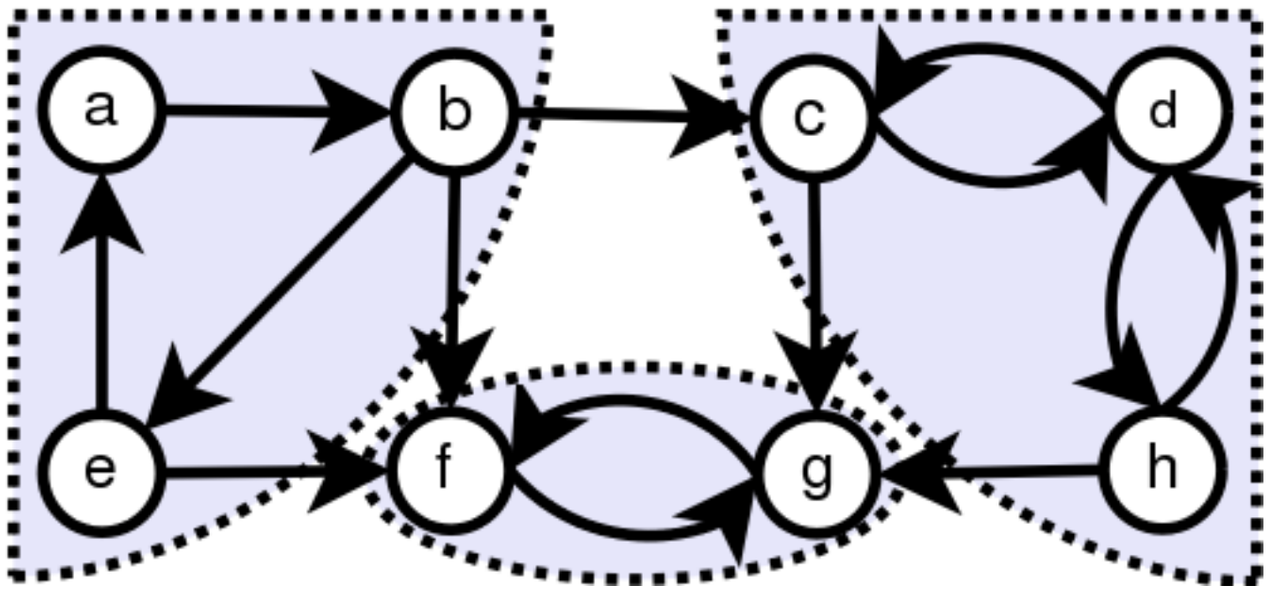
Disconnected network: some of the nodes are unreachable



Connected components

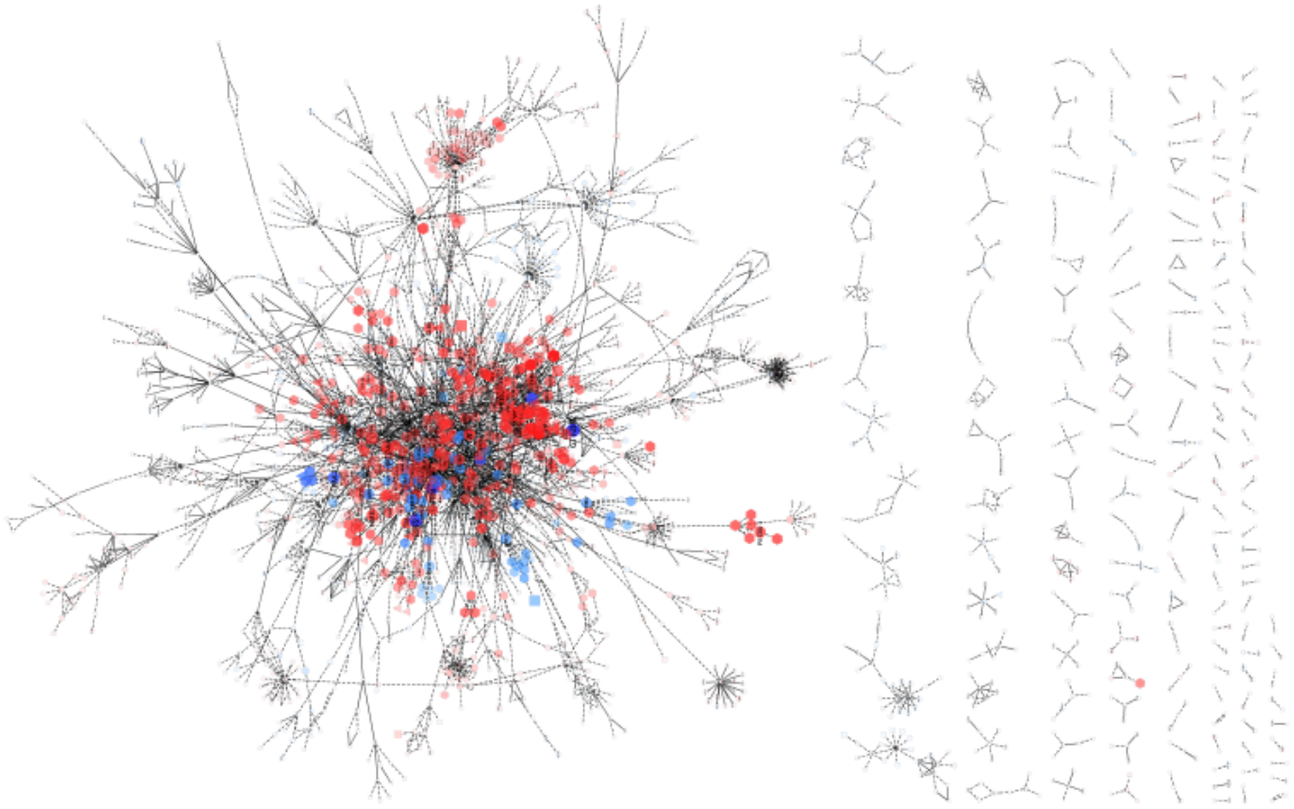
Connected components are those where all nodes of each subgraph are connected.

Weak vs **strong** components



Connected components

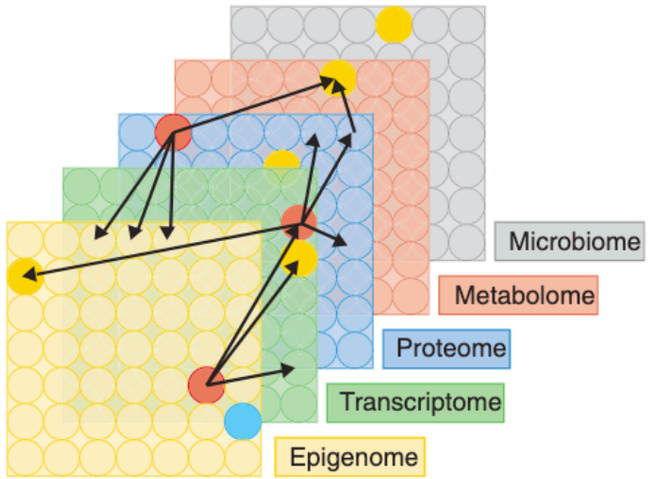
In biological networks, often the most insightful properties come from the **largest connected component**

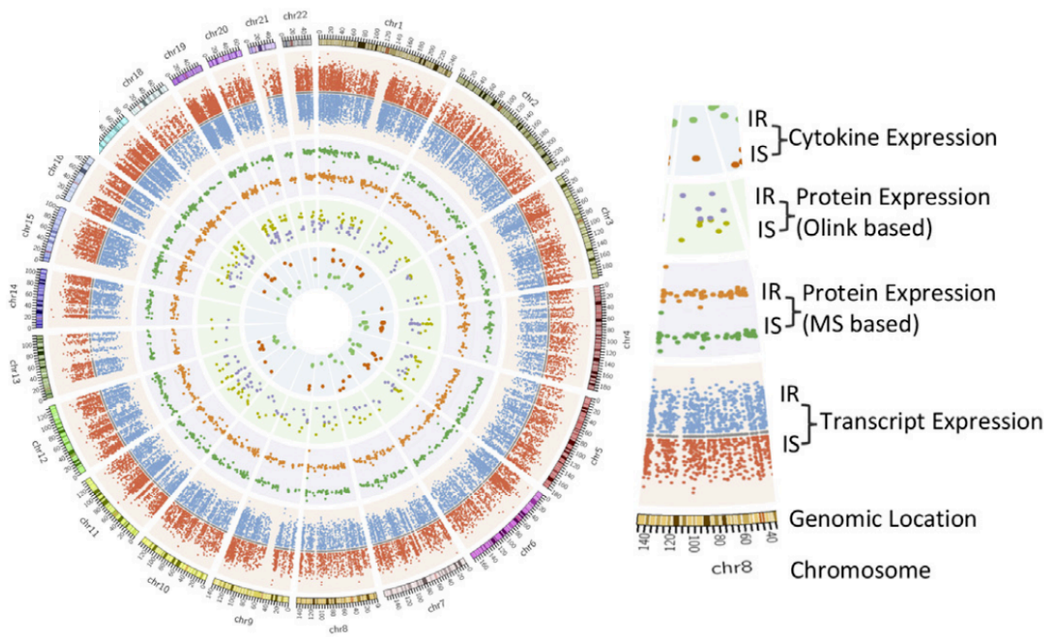


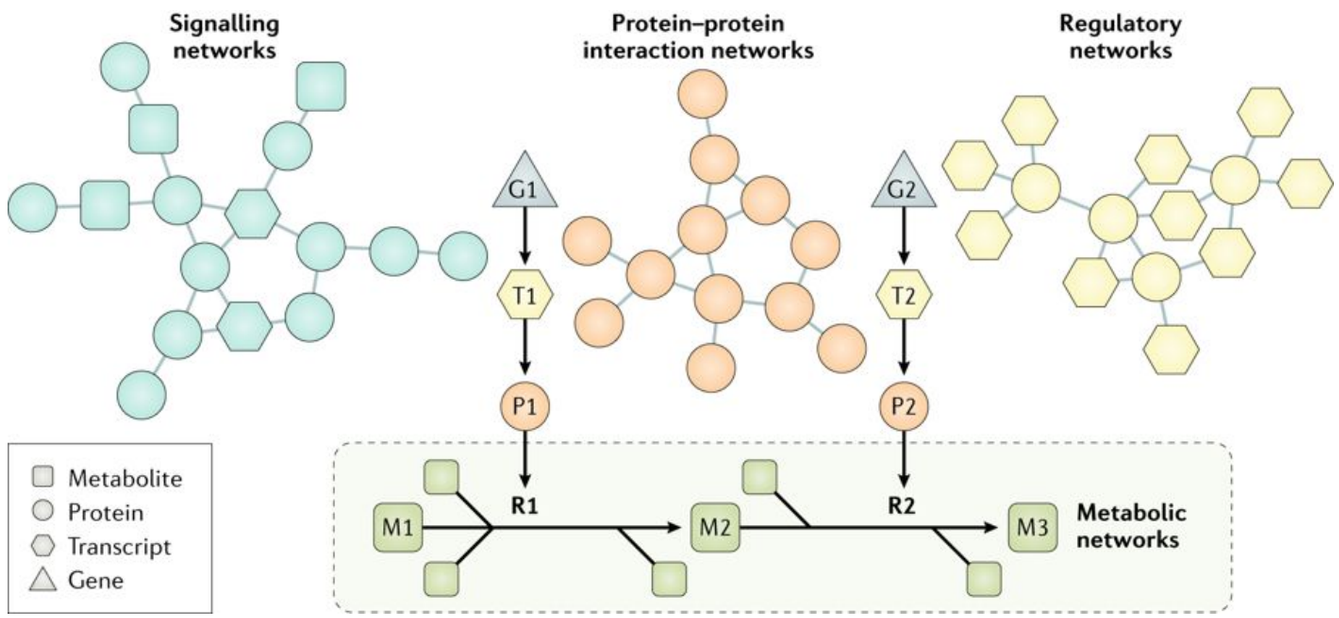
Biological network inference

Building networks

How to go from raw data to a graph-tractable format?







Hasin 2017
Piening 2018
Mardinoglu 2018

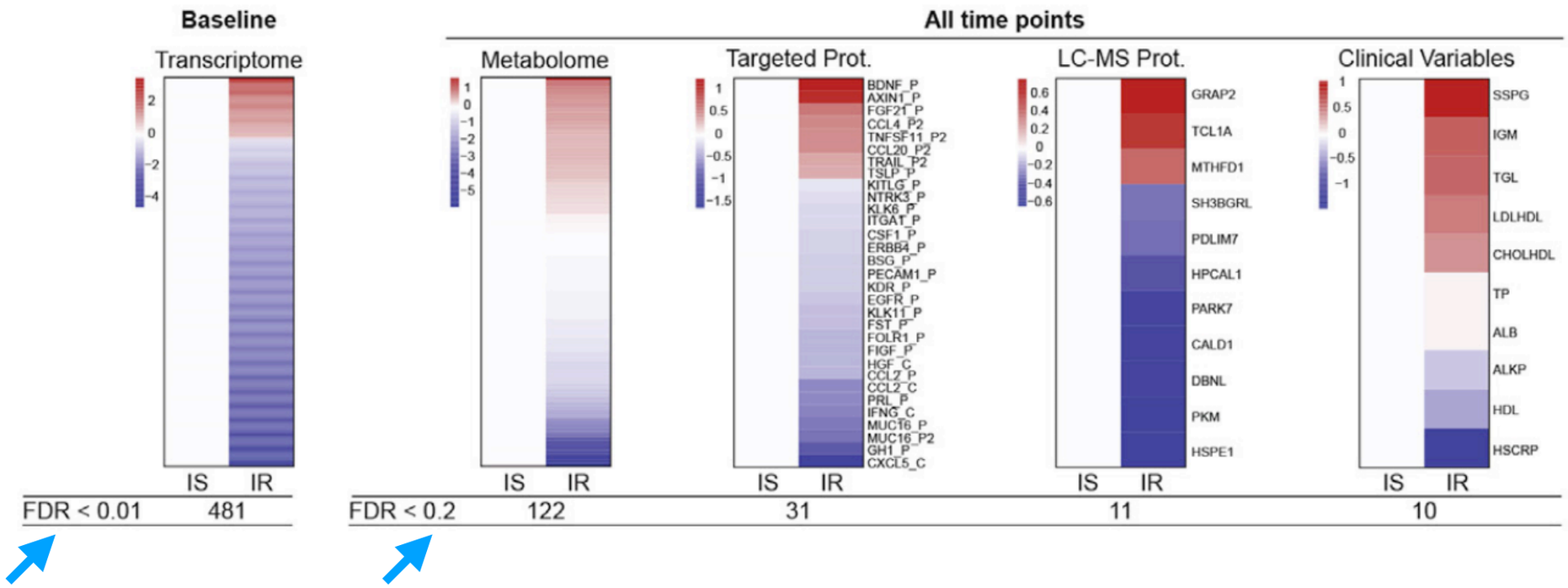
Important considerations

Standard statistics should not be ignored

Importance of power and sample size

Statistical power and significance in high throughput studies

Confounding factors may be a problem: maximise biological signal, minimise technical variation



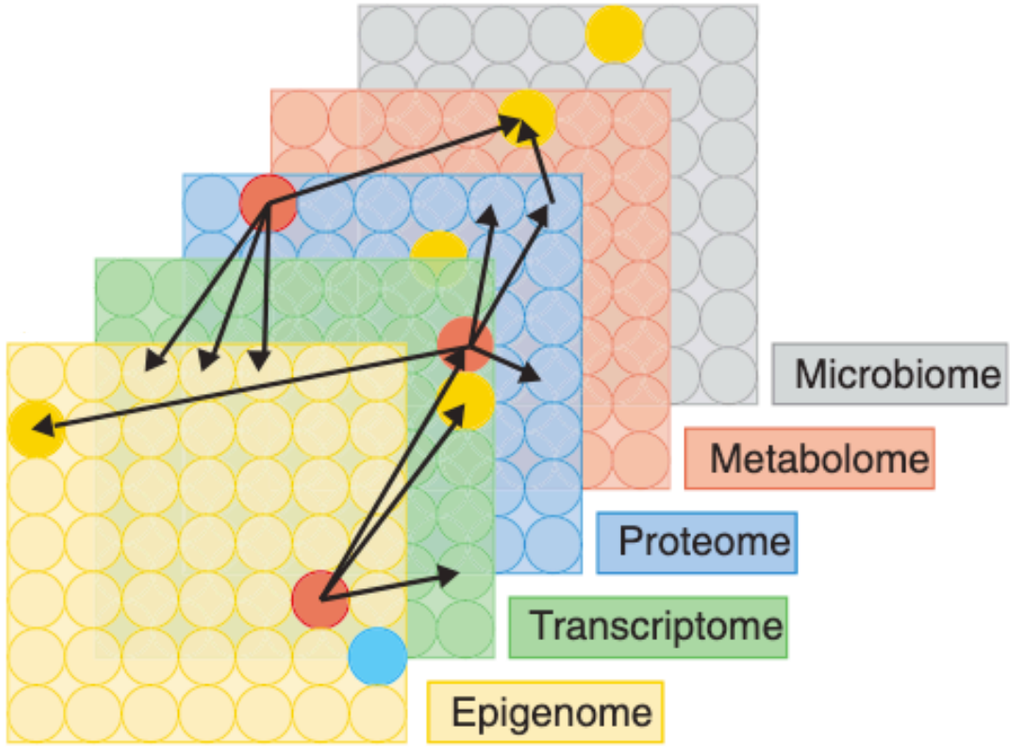
Clarke 2011
Krzywinski 2013
Sham 2014
Nygaard 2016
Piening 2018
other refs as links

Interomic vs Intraomic networks

Networks may be build for individual omics or for their integration

Should I even integrate different omics? What is my biological question?

- Do I want to analyse vertical relationships between features?
- Why integrate omics with different coverage such as transcriptomic and proteomic data
- Do I want to extract functional properties?
- Am I predicting biomarkers?



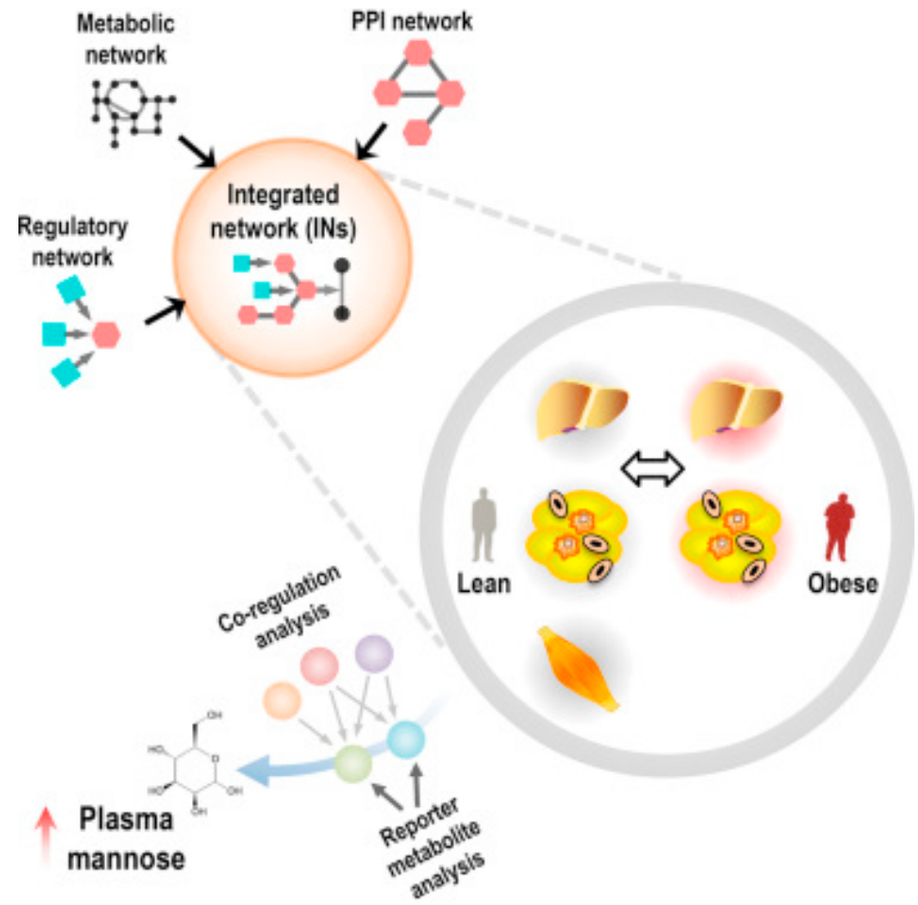
Hasin 2017

Interomic vs Intraomic networks

Multi-modal networks may be generated from different sources

- Transcription-factor - Gene (DNAseq, ChIPseq)
- Gene-gene (Co-expression, PPI, GEMs)
- Gene-metabolite (GEM)
- Metabolite-metabolite (GEM)

Integrated Networks



Different approaches for network inference

1. Feature association
2. K-nearest neighbour graph (k-NNG) construction
3. Latent-factor relationships

Data-driven

No prior info

4. Pathway-based
5. Genome-scale metabolic models

Based on available information

6. Network deconvolution

Filter indirect effects

1. Association analysis

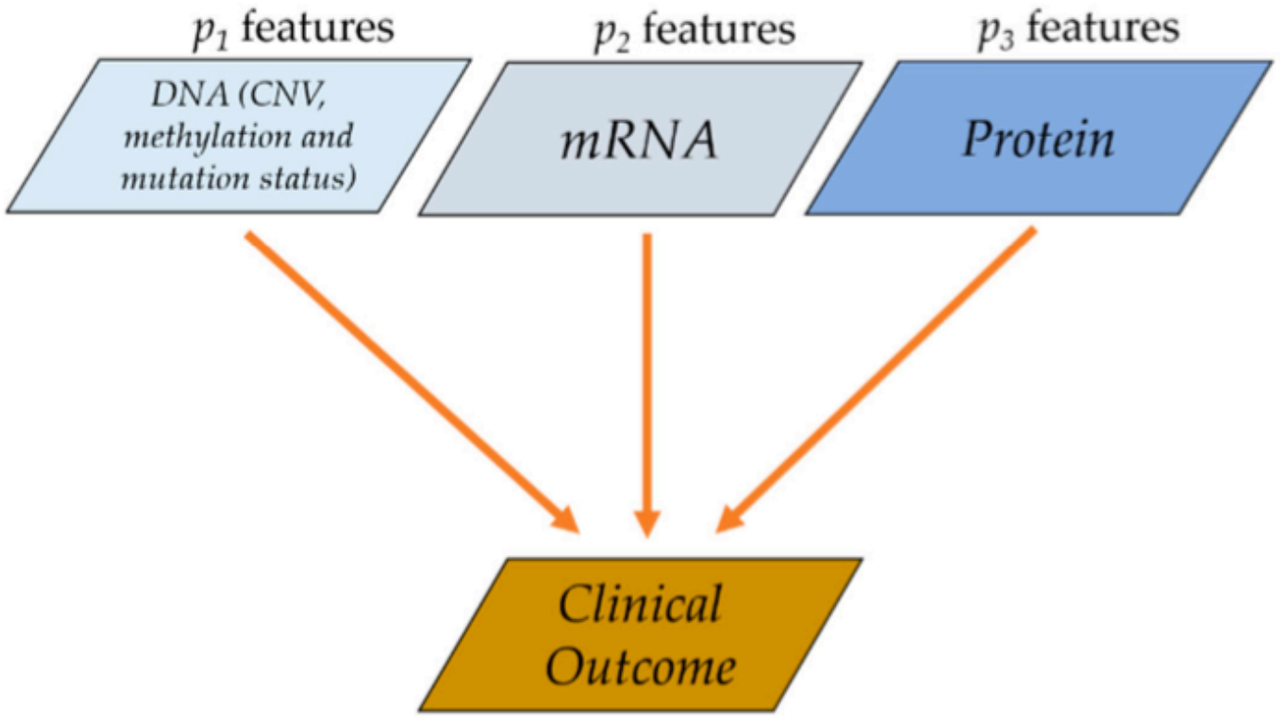
Balanced dataset, standard pre-processing of each omics needed

Normalization *may* be needed to make omic datasets comparable (e.g. standardization)

Common approach: compute correlations between different features

- Spearman
- Pearson

Extend known associations



Wu 2019

1. Association analysis

Easy to interpret

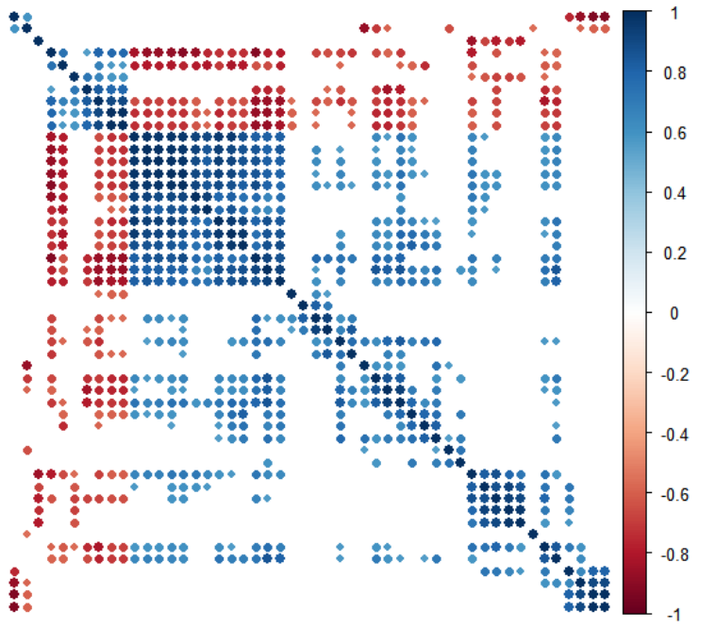
Unweighted vs weighted ($-1 \leq \rho \leq 1$)

Unbalanced networks

Prone to type I errors

- FDR vs Bonferroni
- Correlation coefficient cutoff

Need adjustment to possible confounding factors



1. Association analysis

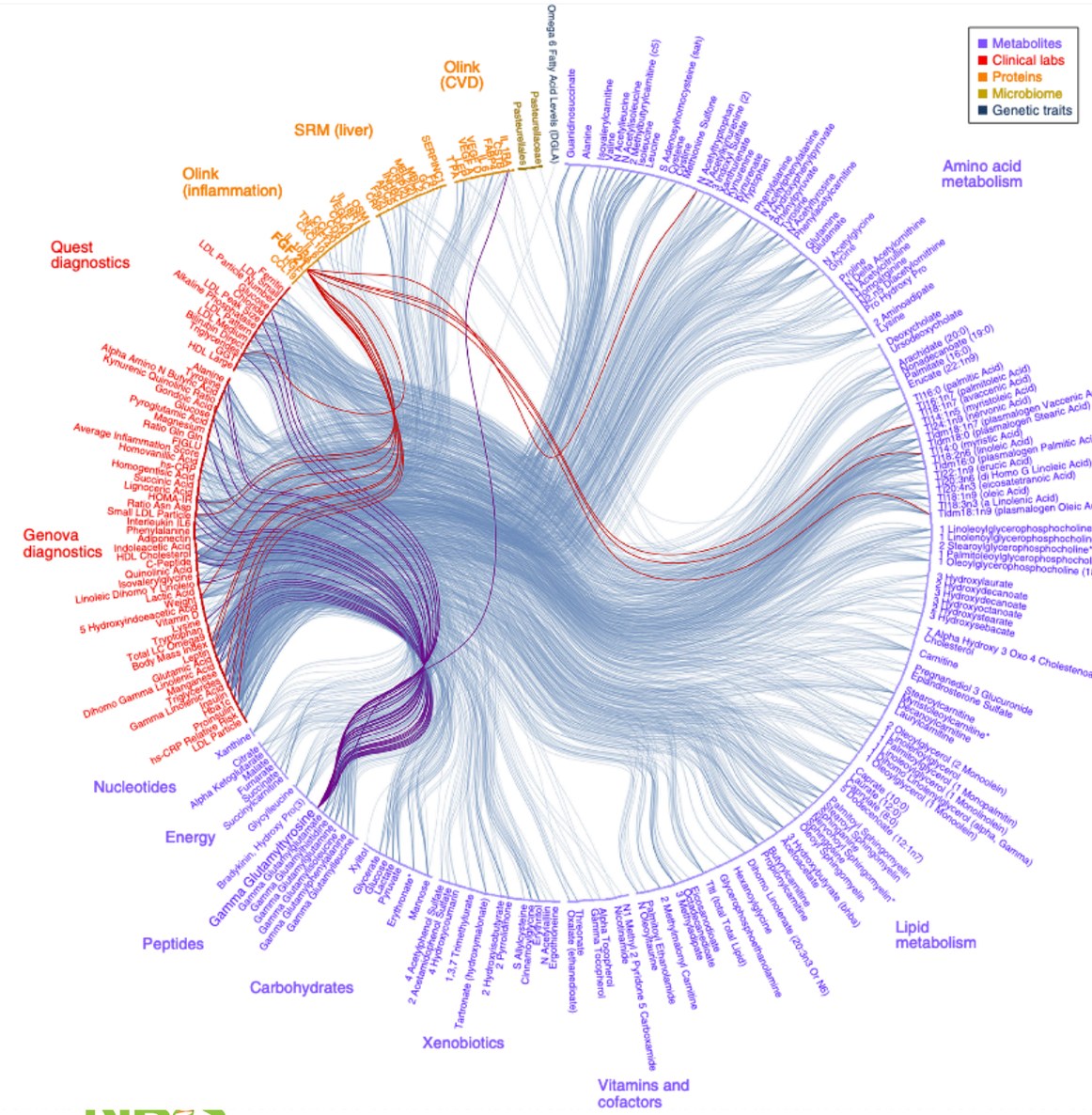
Adjusting for confounding factors: partial correlation analysis

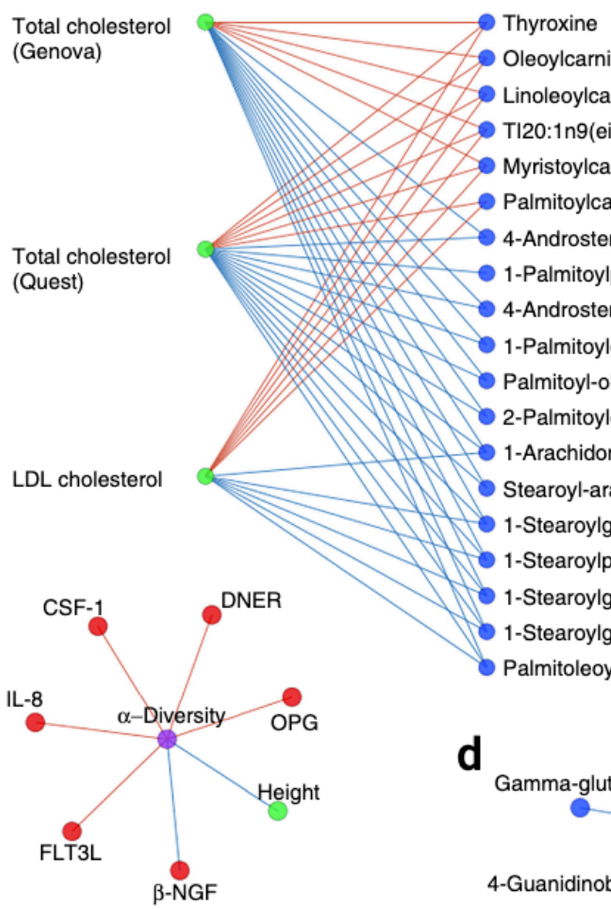
Still considers linear relationship between variables

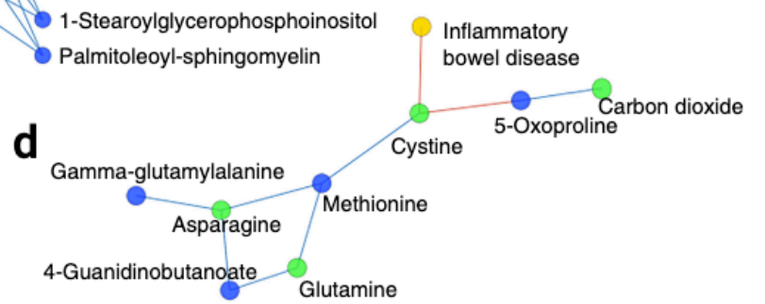
Below:

- gender and age are known confounding factors

- feature regression on confounding factors, followed by correlation on the residuals of each model







2. *k*-nearest neighbour graph

1. For each pair of features (u, v), compute a distance metric:

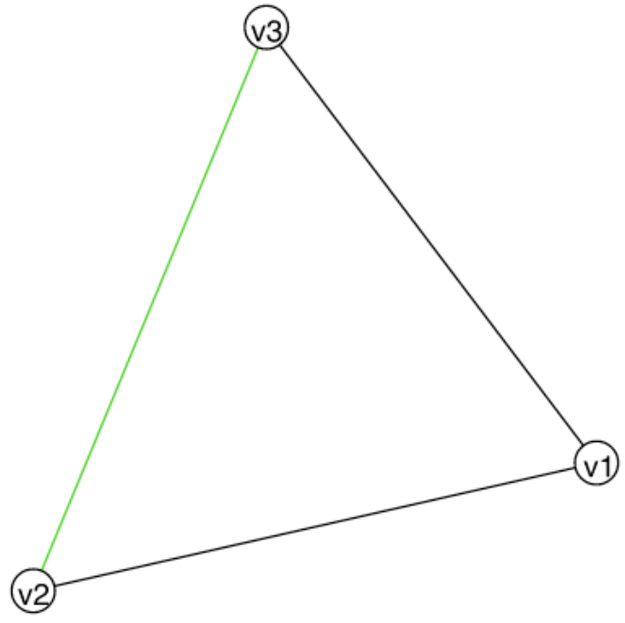
- Correlation

- Euclidean

- Jaccard

- …

2. For each feature, select the *closest k* neighbours



Efficiency (not scalable, compute all neighbours for every node)

Generates well-structured graph

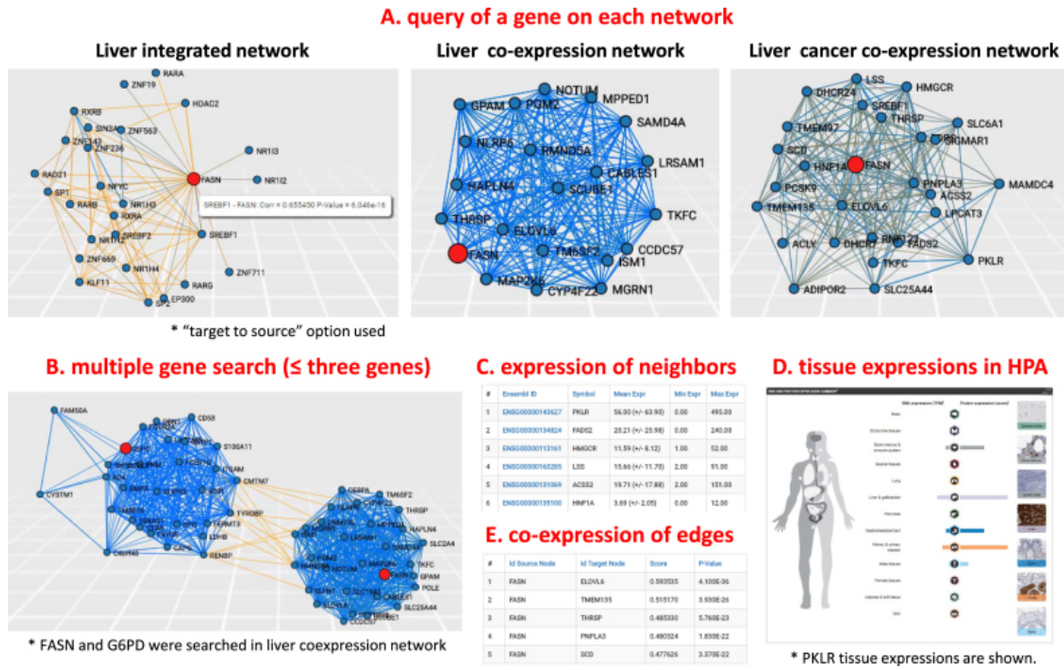
Simple as it reduces the number of features

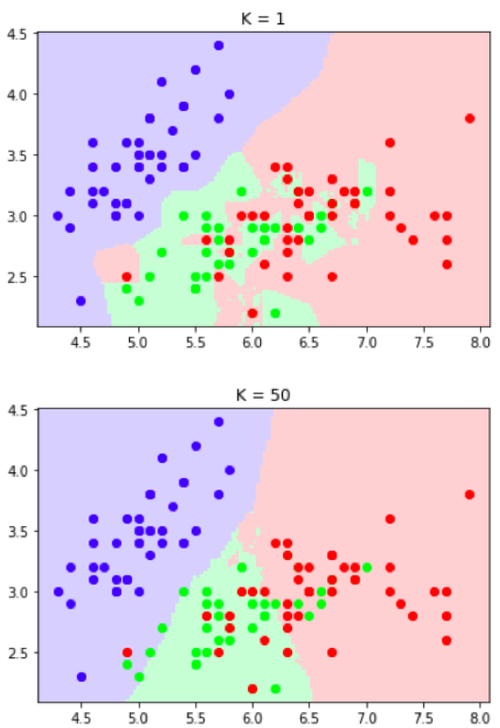
Dong 2011

2. *k*-nearest neighbour graph

High *k* is smooth, but biased (underfitting)

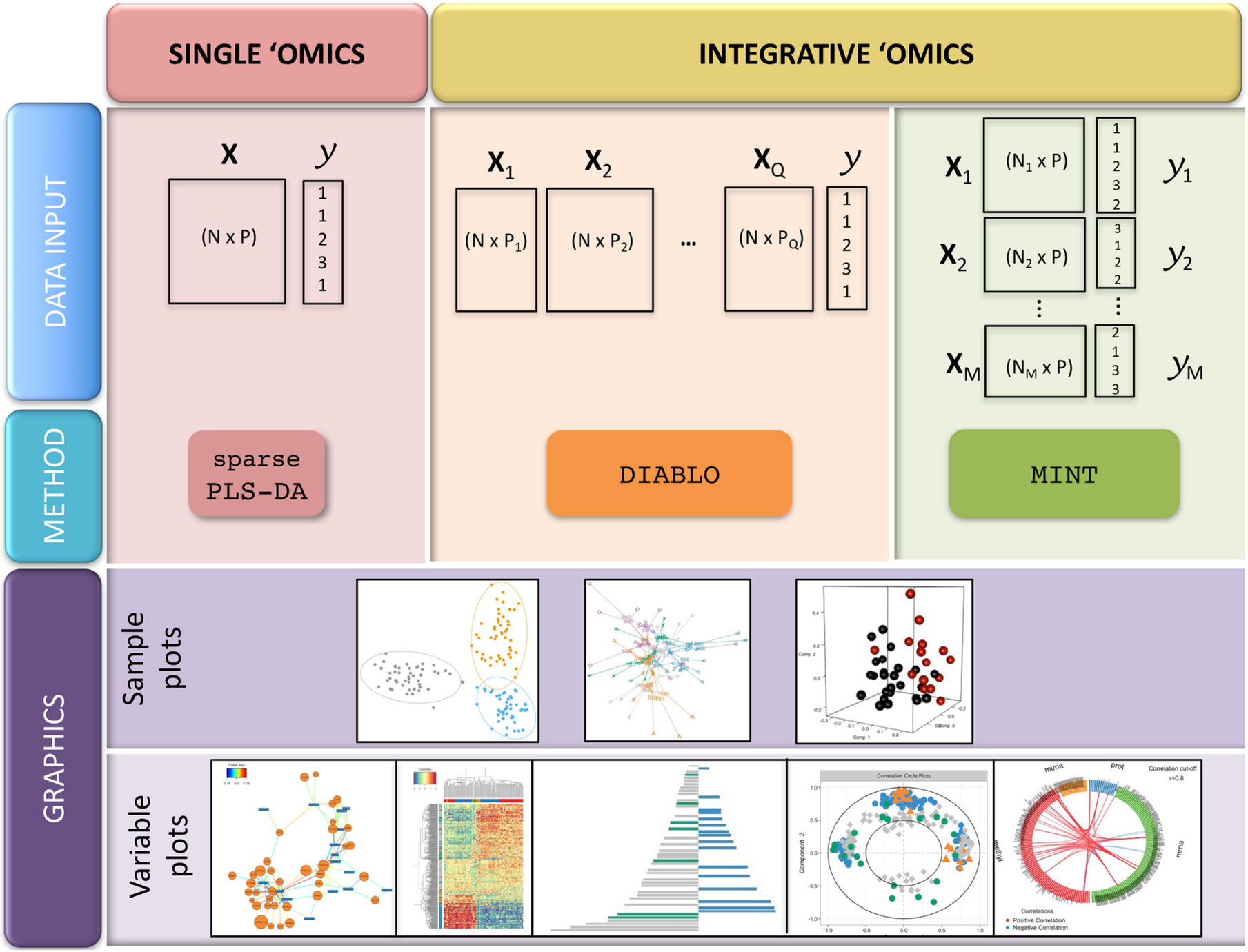
Low *k* is accurate, but noisy (overfitting)





3. Latent-factor extraction

Mixomics



4. Knowledge-based graph creation

Database-derived

- PPI
- TFRN
- Metabolic Atlas
- …

Many reference databases

KEGG

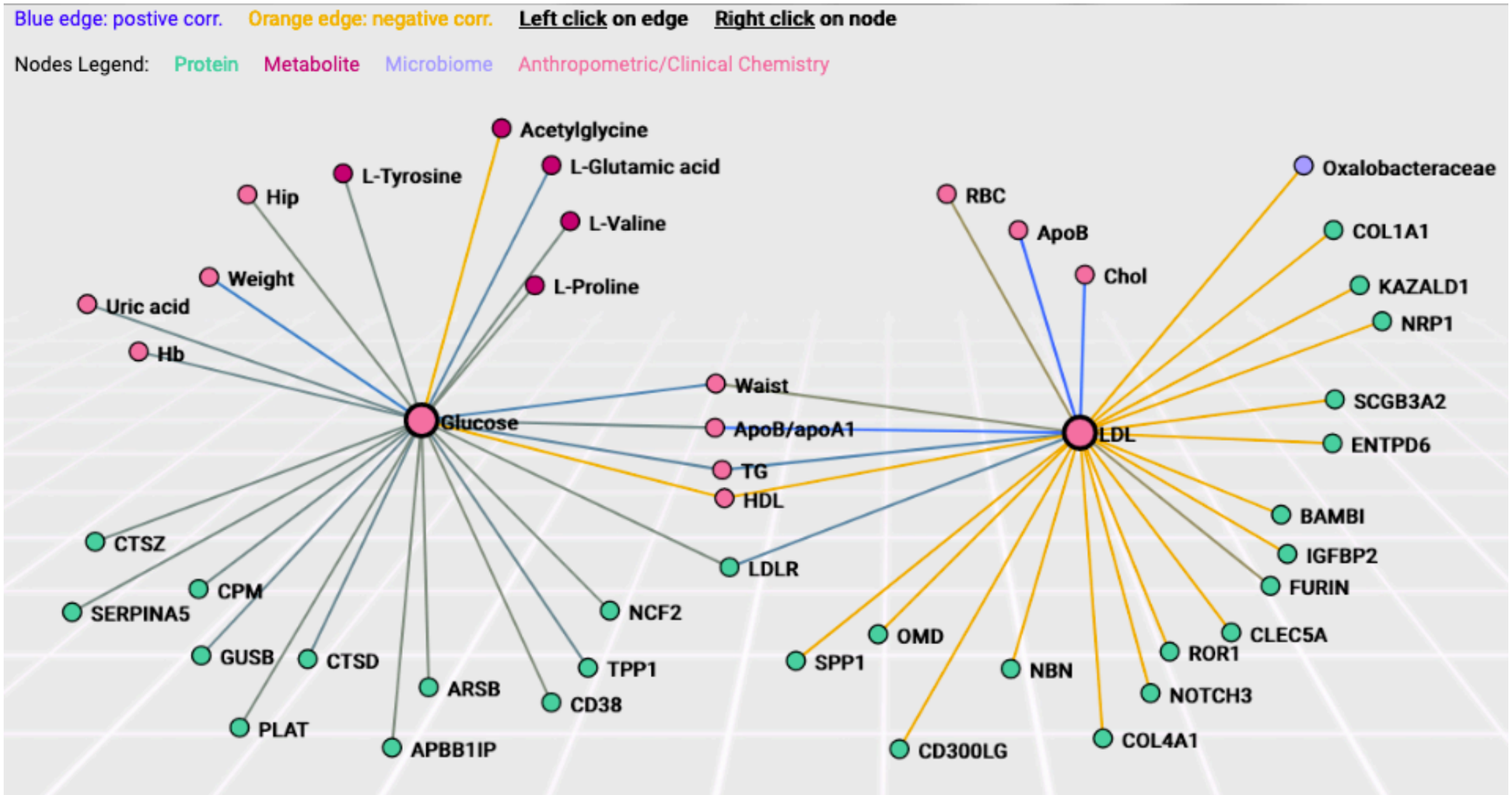
Reactome

WikiPathways

MSigDB

4. Knowledge-based graph creation

Multi-omic biological networks



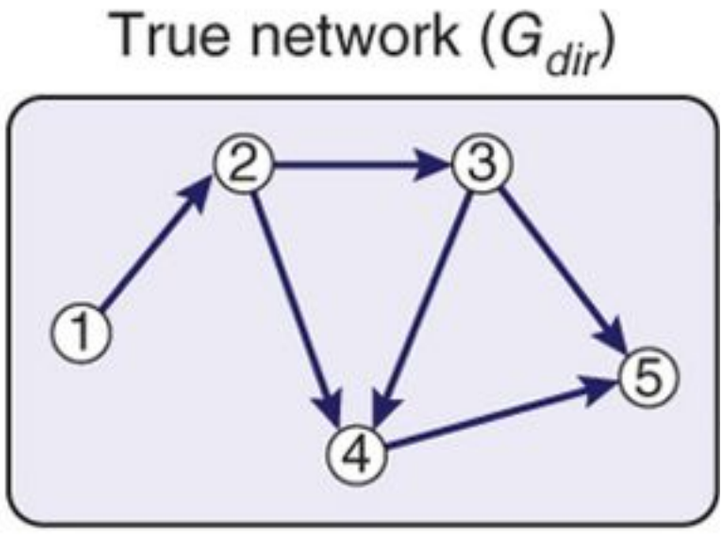
4. Knowledge-based graph creation

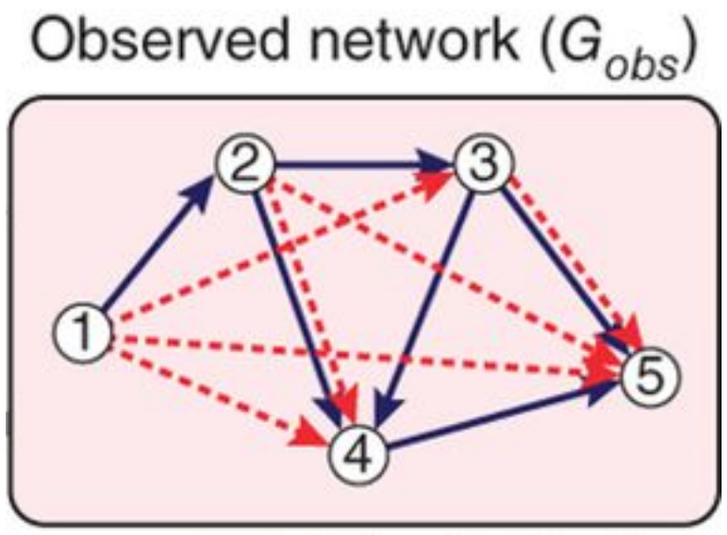
How to overlay your data based on known interactions?

- Filter your predicted interactions based on known information? (intersection)

- Add interactions that are not found in the reference networks?

- Simply consider interactions based on physical presence considering the reference networks?





5. Using genome-scale metabolic models for graph creation

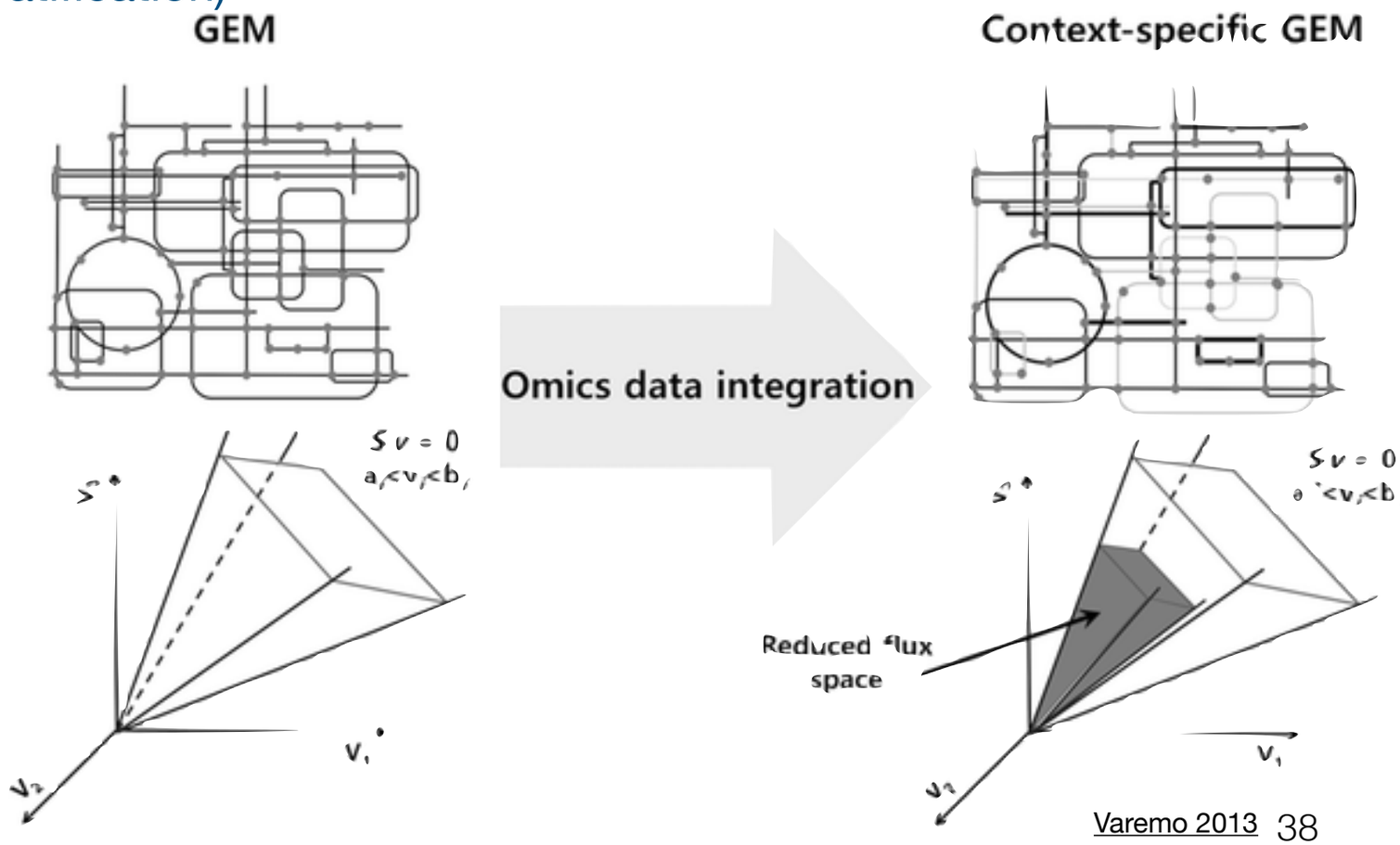
GEMs may be used to find such missing relationships, but there is a coverage issue

The overall strategy follows

1. Integration and flux prediction
2. Compute metabolite-reaction-gene relationships
3. Extract relevant relationships (met-met, gene-gene)

3b. Exclude unnecessary interactions (e.g. cofactors)

4. Downstream analysis (e.g. topology, stratification)

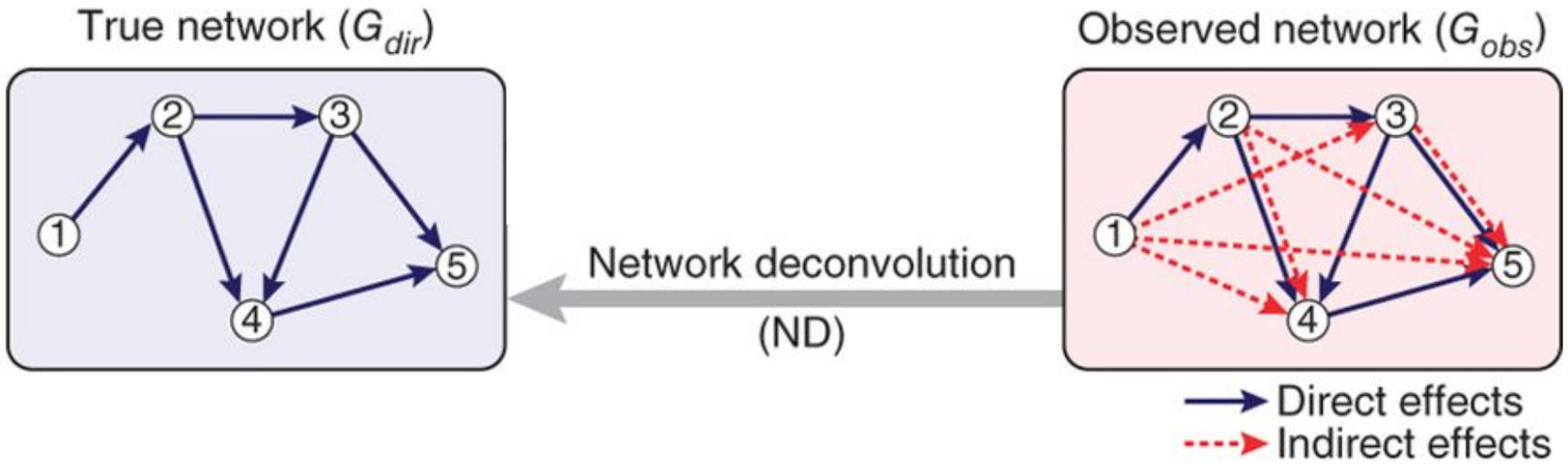


6. Network deconvolution

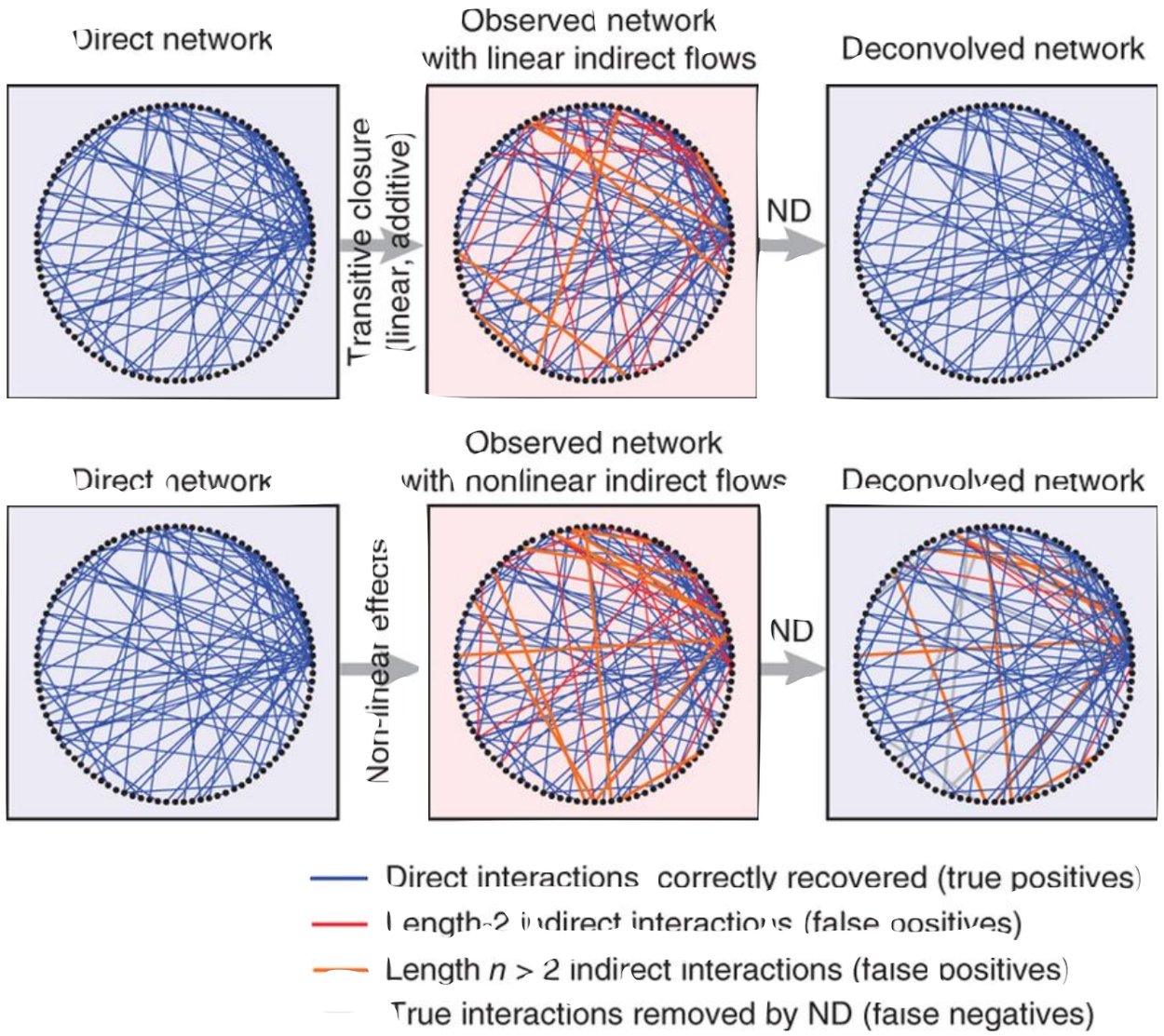
Biology is ***noisy***, which may result in edges that are not true

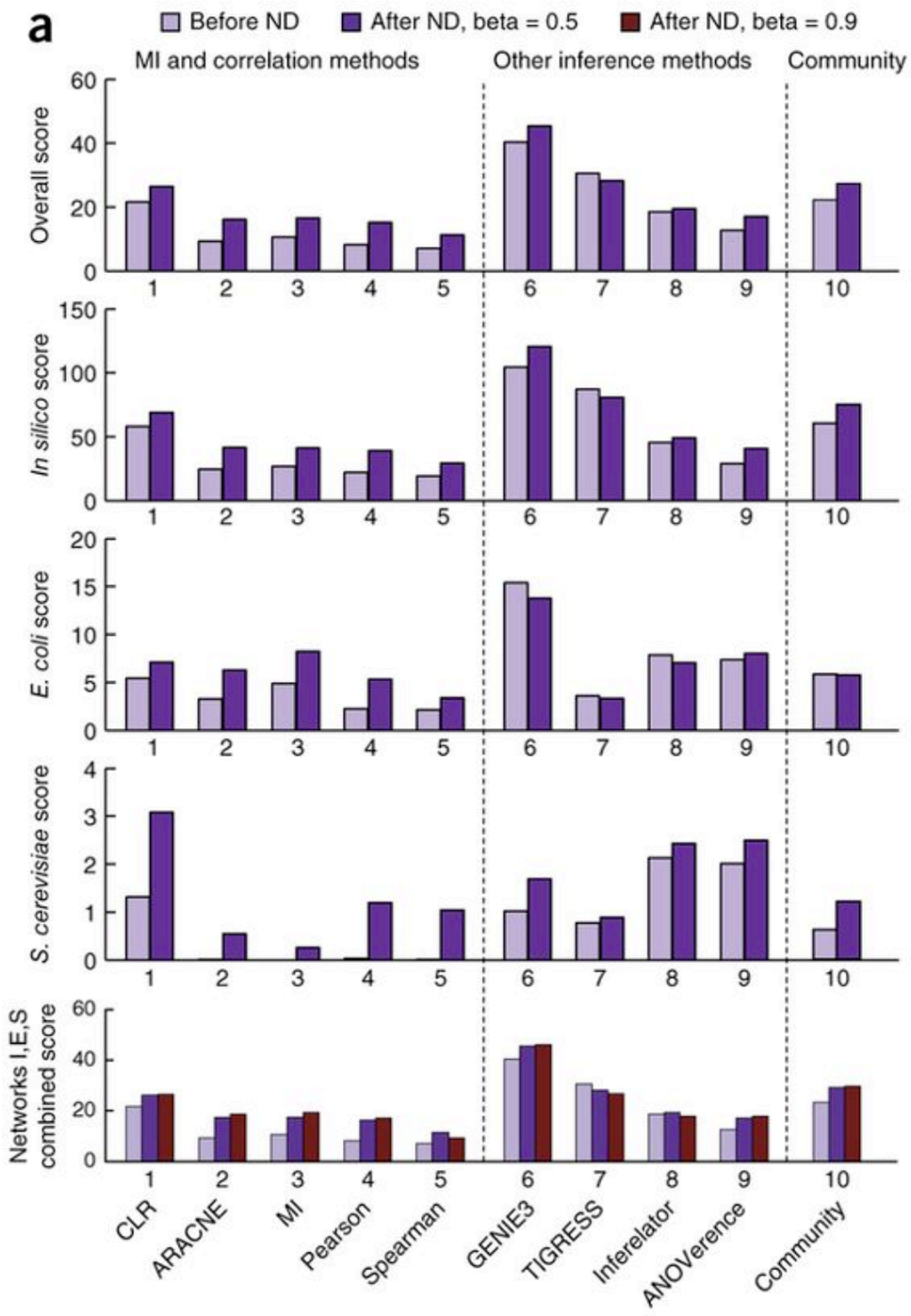
- 1 → 2
- 2 → 3
- 1 ⇢ 3

Direct and indirect effects



5. Network deconvolution





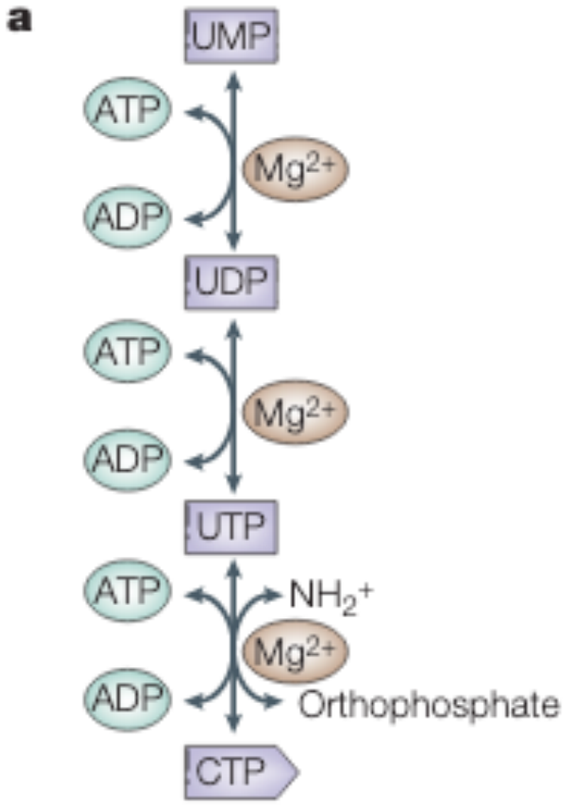
Key network properties

1. Introduction
2. Terminology
3. Network construction
4. **Key properties**
5. Community analysis
6. Visualization
7. Workshop

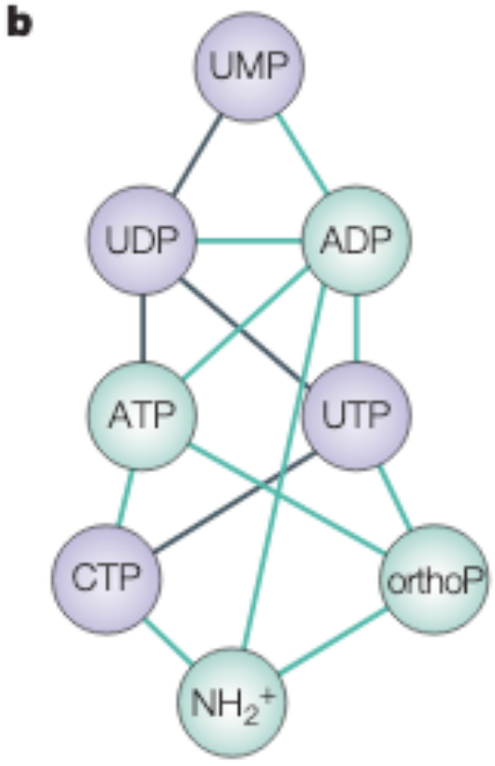
1. Network representations

Representations of a metabolic network: pyrimidine metabolism

Metabolism



Graph representation: metabolites and co-factors



metabolite-metabolite association

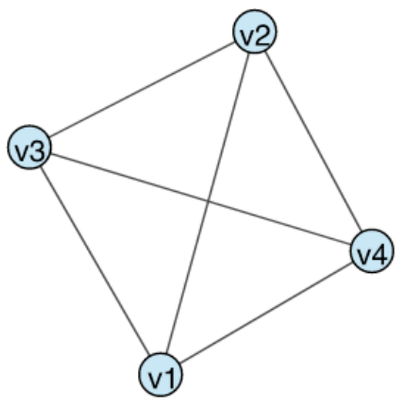
Other representations: Protein-Protein, Protein-Metabolite, …

2. Network density

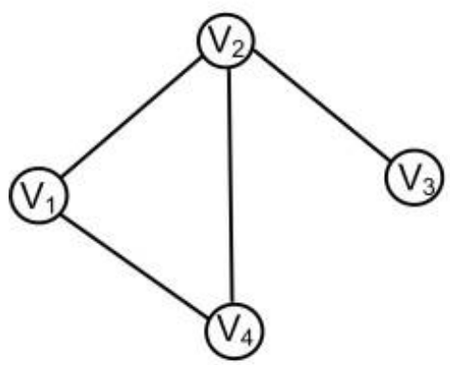
A **dense graph** is a graph where the number of edges approximates the maximum possible number of edges for the given node number.

$$0 \leq D \leq 1$$

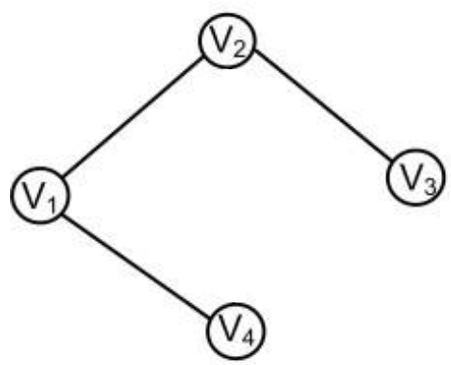
Higher density indicates higher associations in the network, which implies lower resilience to changes.



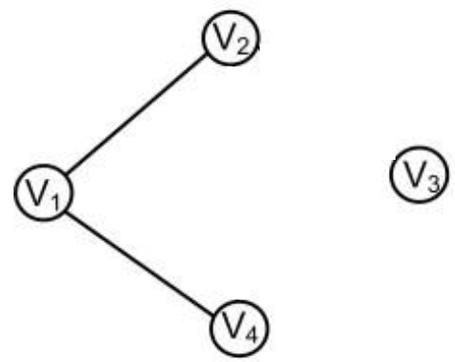
$D = 1$



$D \approx 0.67$



$D = 0.5$



$D \approx 0.33$

2. Biological network density

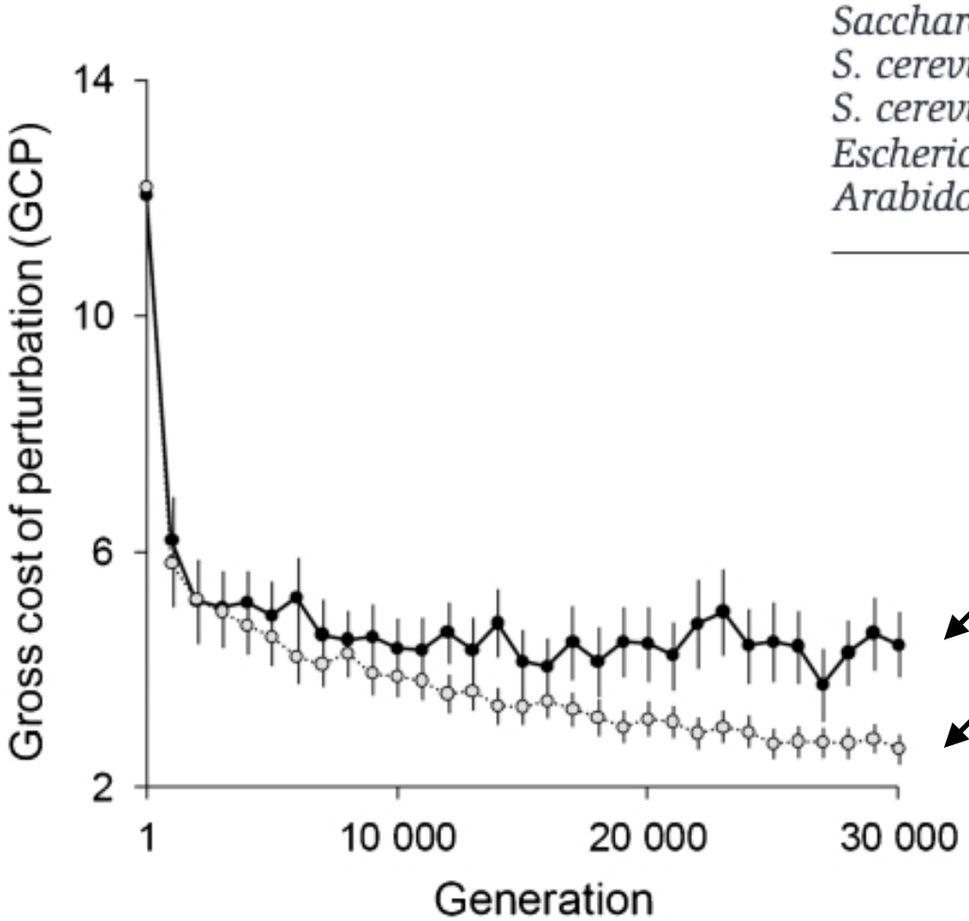
Evolutionary analysis of biological networks indicates general sparsity

Network structure must balance robustness to mutation, stochasticity and environmental queues

Sparse networks show higher robustness when accounting for costs and benefits of complexity

Table I Biological networks are sparsely connected

Organism	Interactions	Genes	D	K
Drosophila melanogaster	29	14	0.148	2.07
D. melanogaster	45	25	0.072	1.8
Sea urchin	82	44	0.0065	1.86
Saccharomyces cerevisiae	1052	678	0.0023	1.55
S. cerevisiae	3969	2341	0.0007	1.7
S. cerevisiae	106	56	*0.0338*	1.9
Escherichia coli[a]	578	423	0.0032	1.37
Arabidopsis thaliana[b]	18 625	6760	0.0004	2.75



dense network (density 0.9)

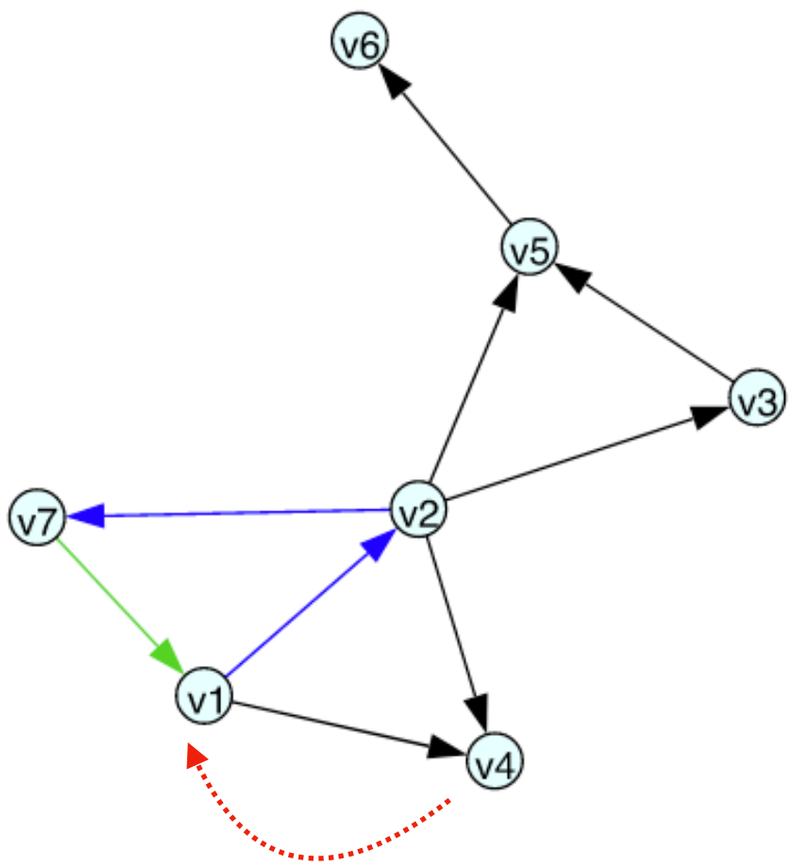
sparse network (density 0.1)

Leclerc 2008

3. Paths

Distance between nodes is measured in path length

In directed graphs, the shortest path between $(a, b) \neq (b, a)$

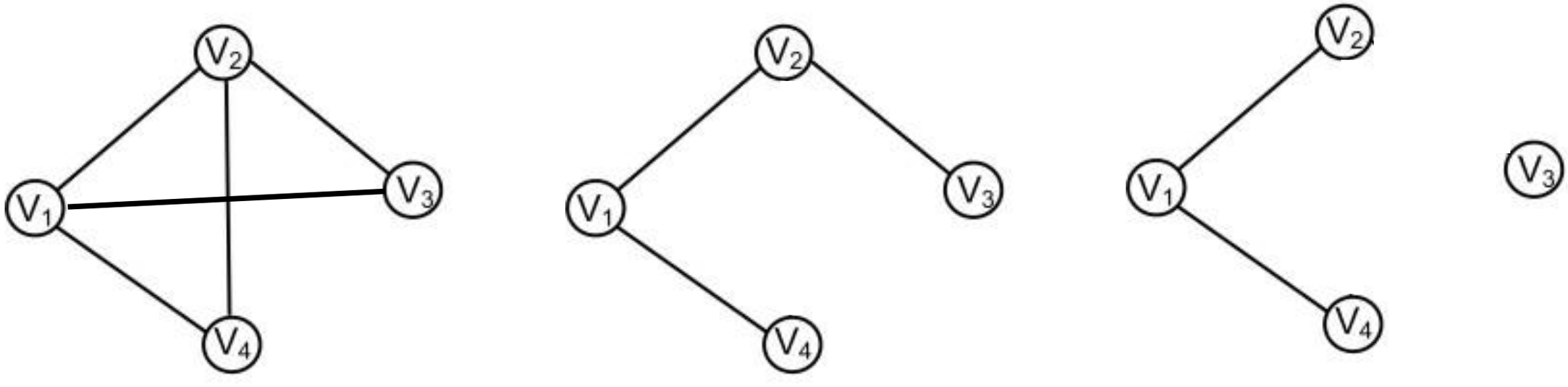


	v1	v2	v4	v3	v5	v7	v6
v1	0.0	1.0	1.0	2.0	2.0	2.0	3.0
v2	2.0	0.0	1.0	1.0	1.0	1.0	2.0
v4	inf	inf	0.0	inf	inf	inf	inf
v3	inf	inf	inf	0.0	1.0	inf	2.0
v5	inf	inf	inf	inf	0.0	inf	1.0
v7	1.0	2.0	2.0	3.0	3.0	0.0	4.0
v6	inf	inf	inf	inf	inf	inf	0.0

4. Connectivity

Node connectivity $\kappa(G)$: minimum number of **nodes** whose removal renders the network disconnected

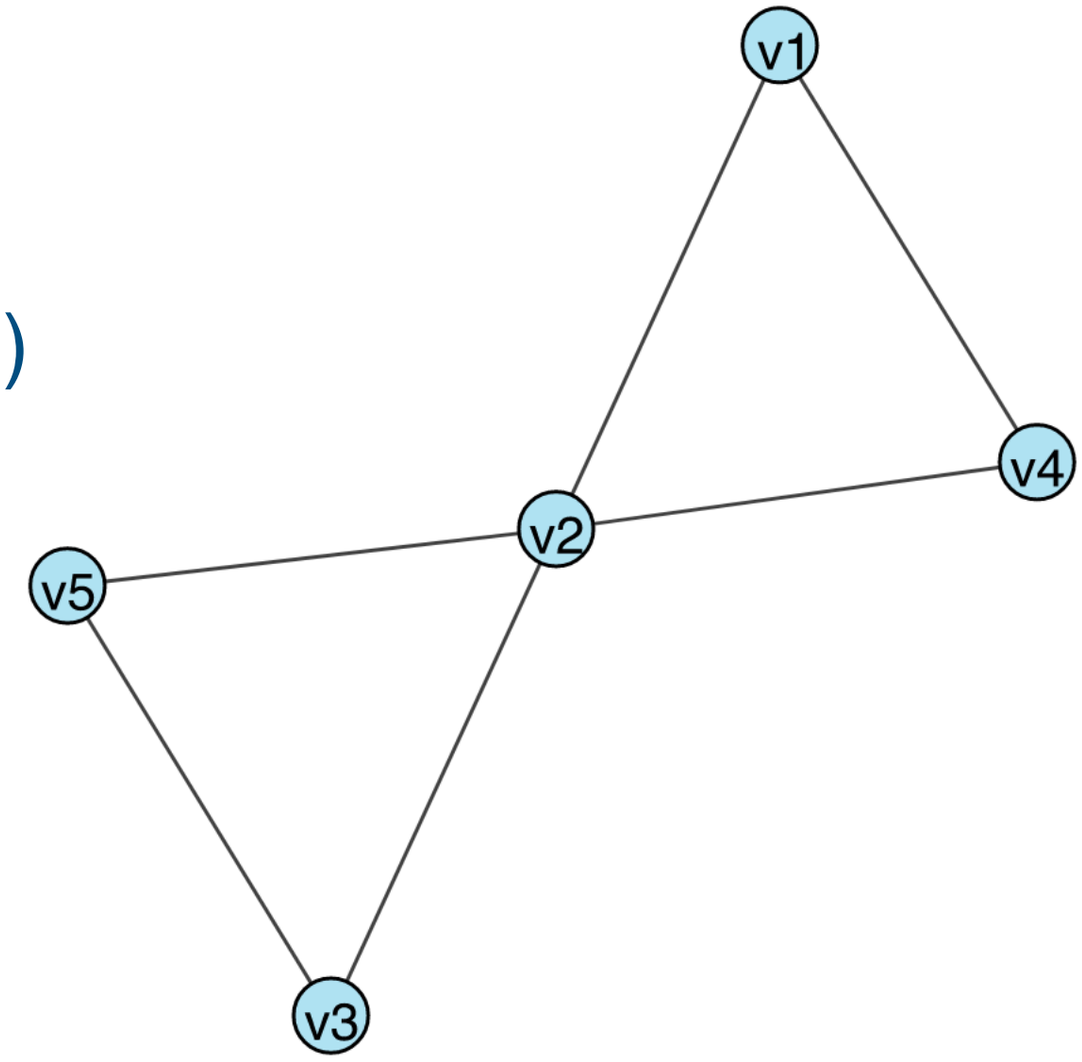
Edge connectivity $\lambda(G)$: minimum number of **edges** whose removal renders the network disconnected



4. Connectivity

$\kappa(G) = 1$; **cut:** v_2

$\lambda(G) = 2$; **bridge:** ((v_2 , v_1) & (v_2 , v_4))



Local connectivity may also be computed for any given pair of vertices

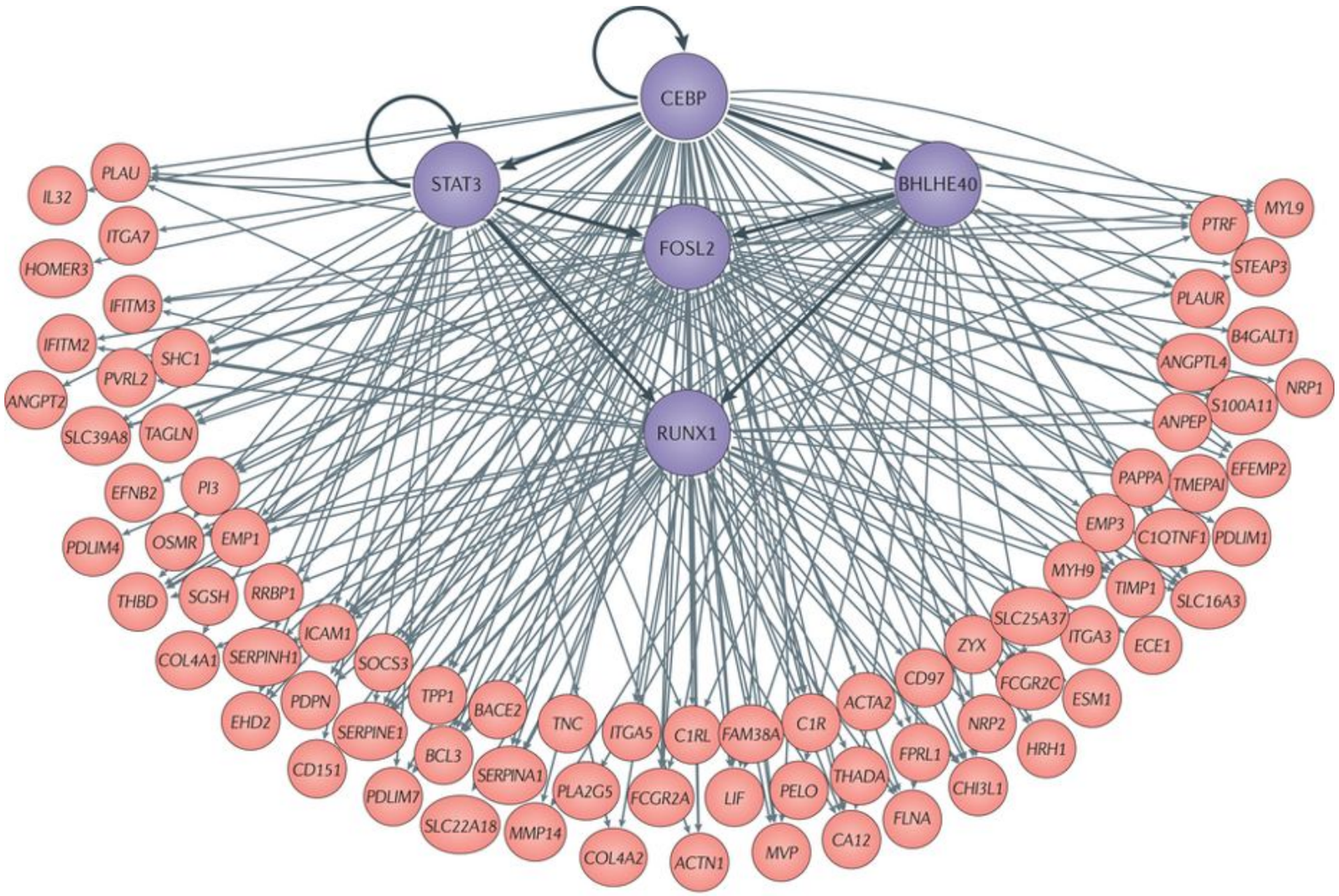
(e.g. v3,v1: v2 and associated edges)

5. Centrality

Indicate the most central nodes in a network

Central nodes **<u>may</u>** act as **hubs** and have important roles

Example: Transcription Factor Master Regulators



Nature Reviews | Cancer

5. Centrality

There are many different measures of centrality:

- **Degree**
- **Eccentricity**
- Closeness
- Betweenness
- Eigenvector
- Katz
- PageRank
- Percolation
- Cross-clique

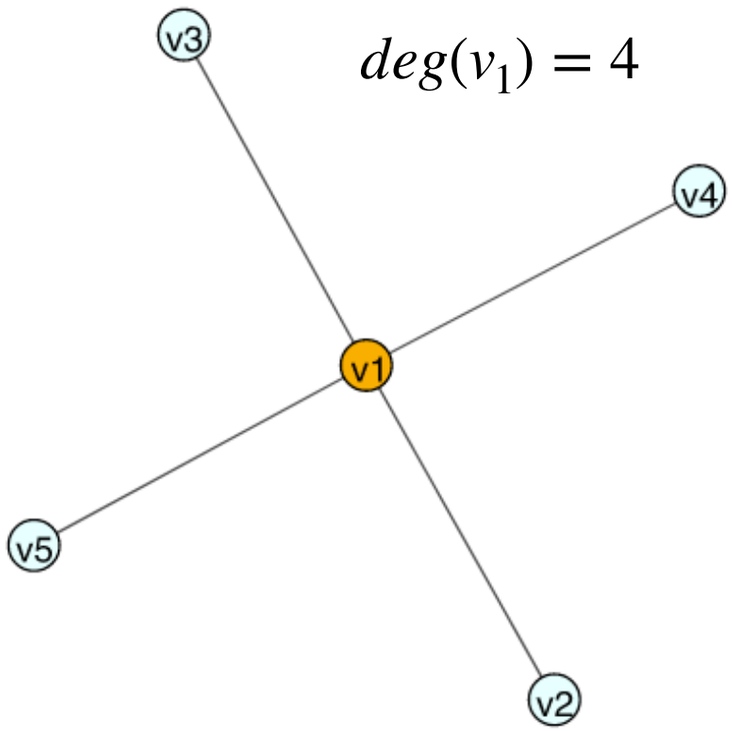
...

5. Centrality: degree centrality

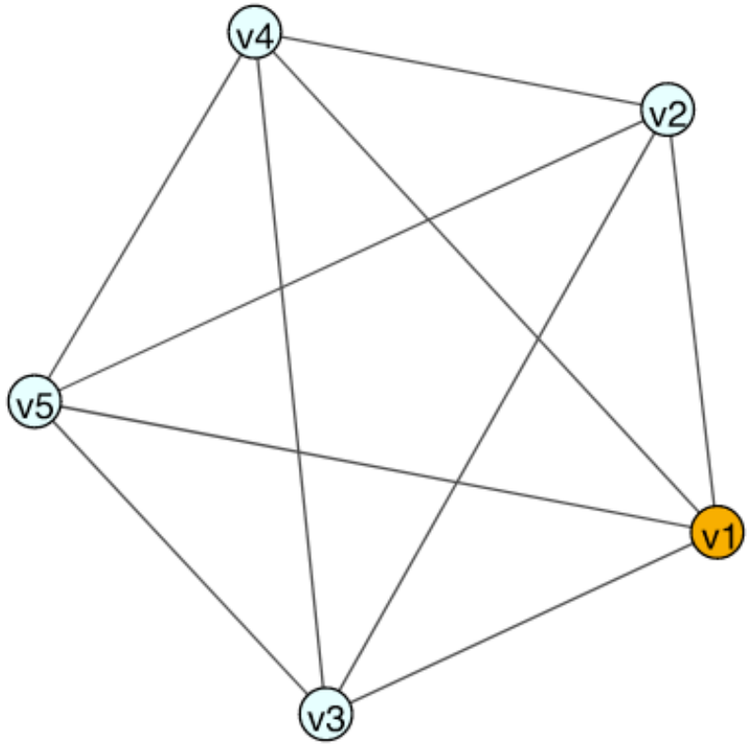
Degree indicates the number of connections with a node

$$d(v) = |N(i)|$$

where *N(i)* is the number of 1st neighbours of a node.



$deg(v_1) = 4$



$deg(v_1) = 4$

5. Centrality: degree centrality

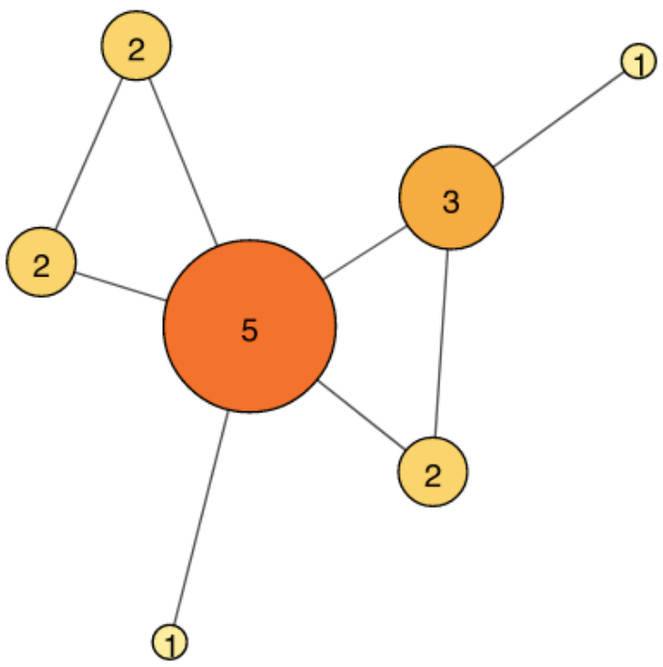
Undirected networks vs directed networks

In-degree vs **Out-degree**

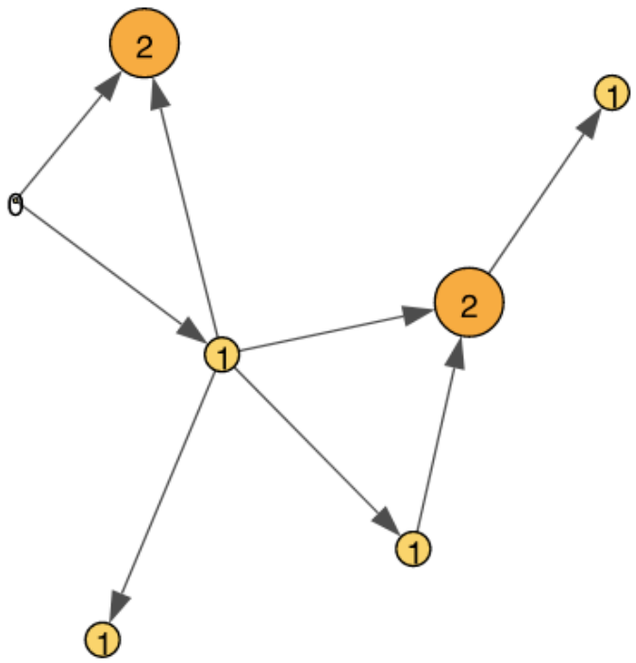
$$C_D(v_i) = \sum_{j=1}^{N} e_{ij}$$

Numbers indicate degree:

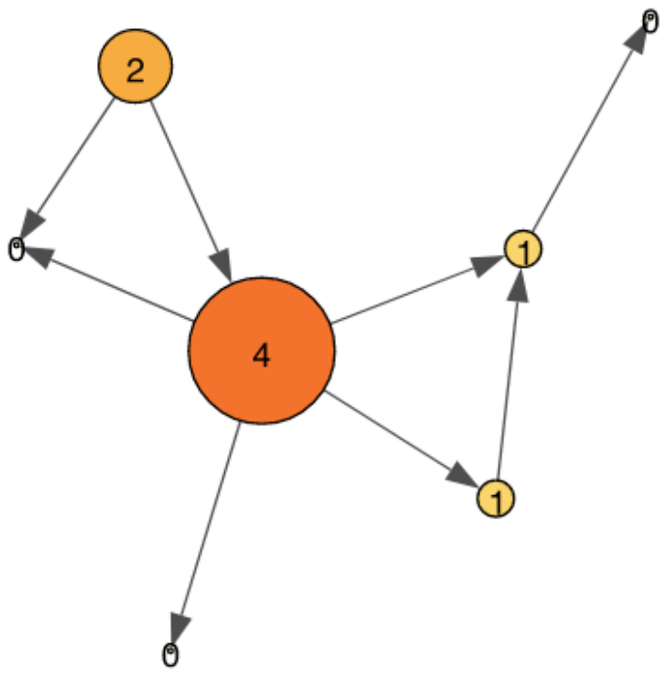
Undirected



In-degree



Out-degree



5. Centrality: degree centrality

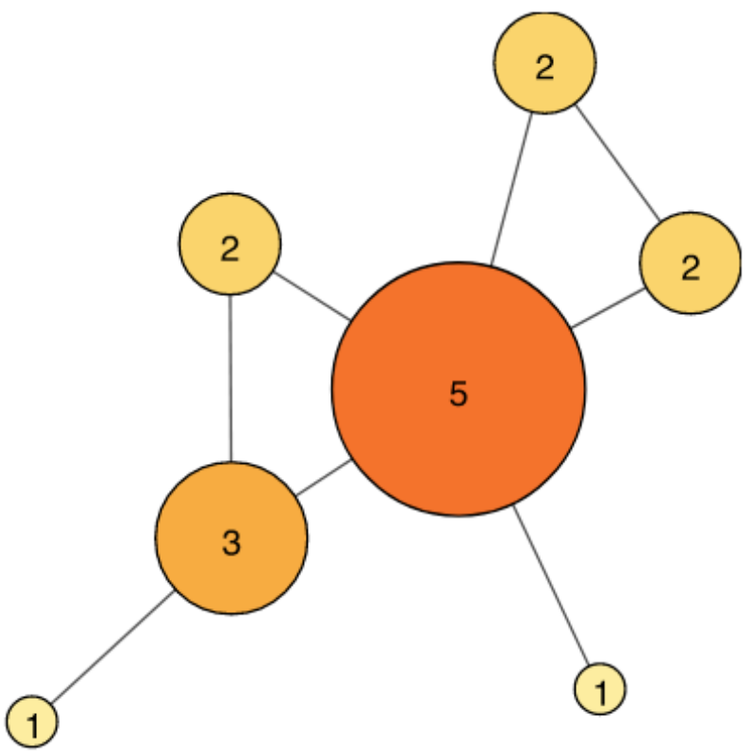
Degree centrality

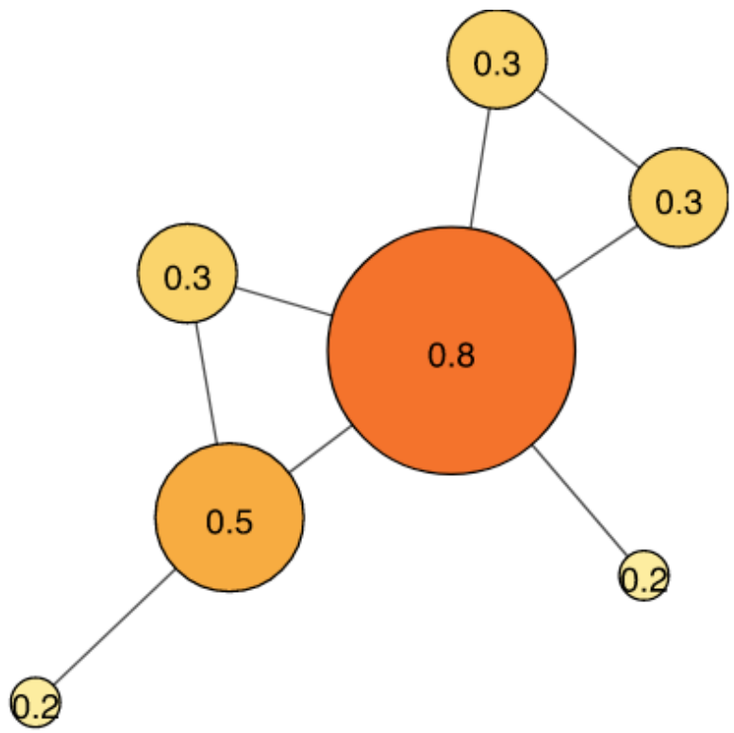
$$C_D(v_i) = \sum_{j=1}^{N} e_{ij}$$

Normalized degree centrality

$$C_D(v_i) = \frac{\sum_{j=1}^{N} e_{ij}}{N-1}$$

Centrality normalization allows for comparison between networks of different sizes

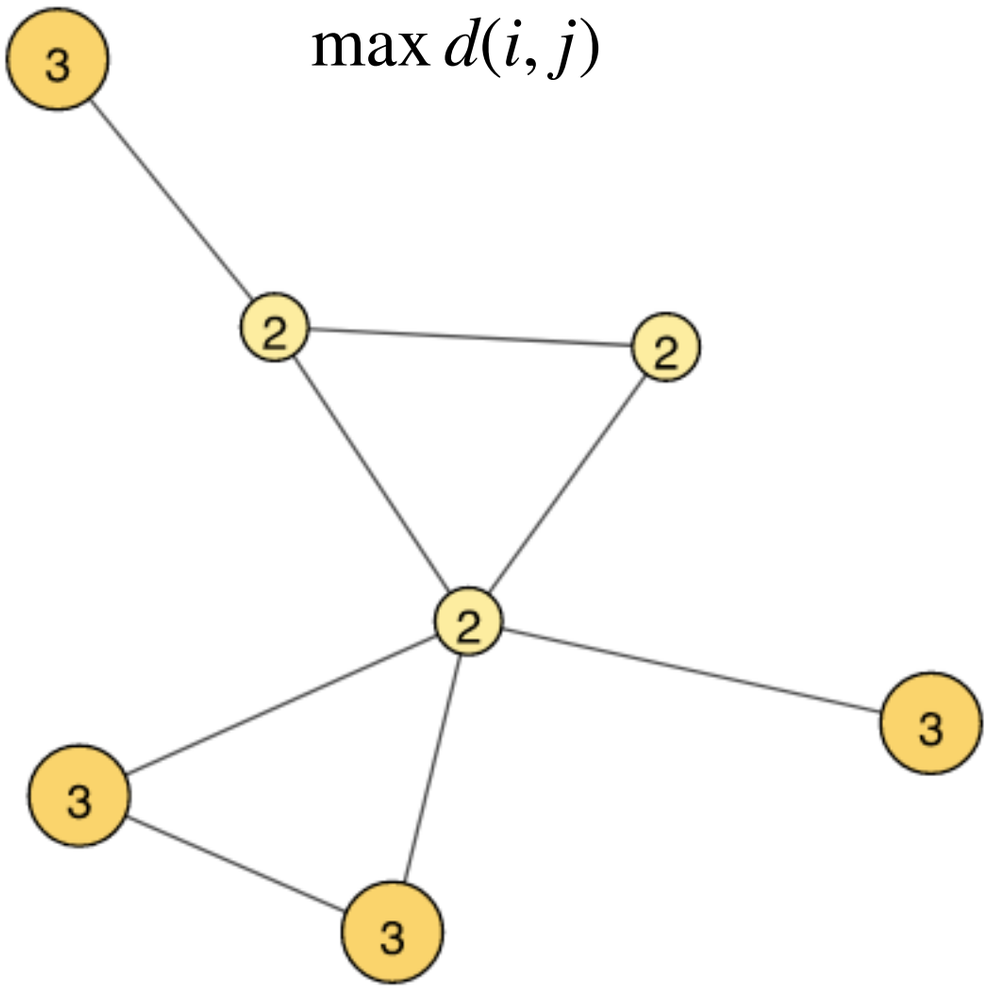




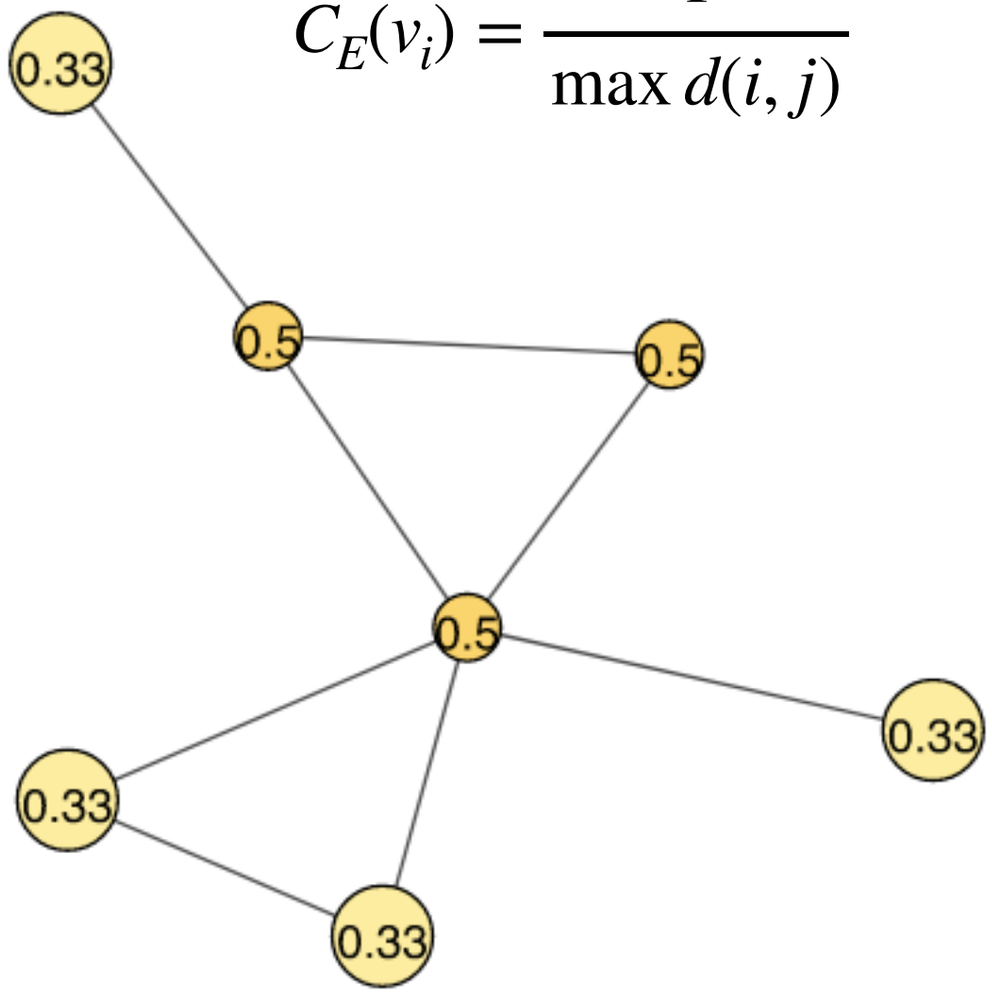
5. Centrality: eccentricity centrality

Eccentricity considers a node's max path to all other nodes

$$\max d(i, j)$$



$$C_E(v_i) = \frac{1}{\max d(i, j)}$$



5. Centrality: limitations & influence

Node centrality does not necessarily imply **importance**

How to tackle this?

1. Complement with experimental observations
2. Compute multiple metrics and summarise joint observations
3. Measure **information transmission** rather than *connectiveness*

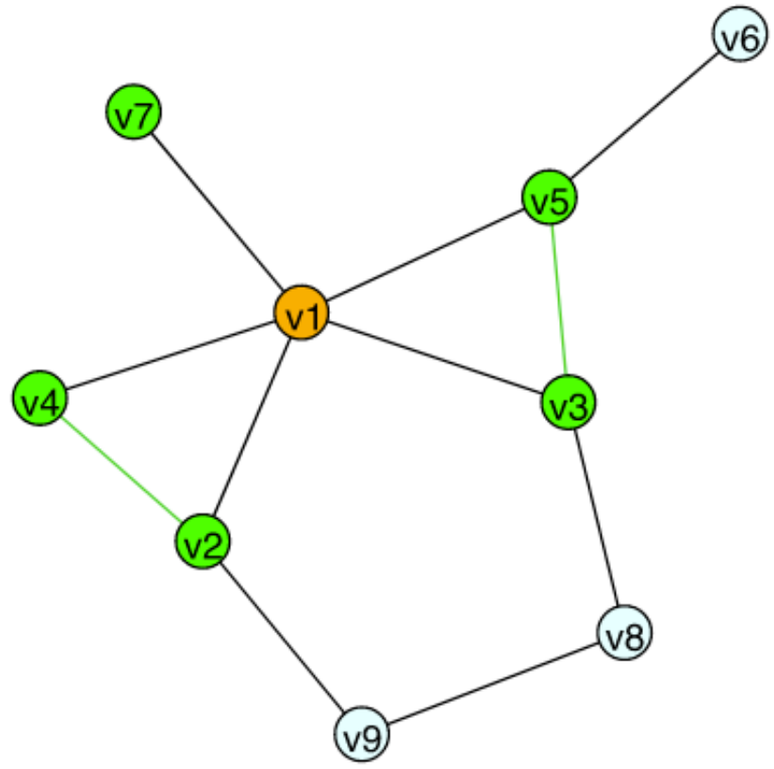
- **Accessibility**
- **Dynamic influence**
- **Impact**
- **Expected force**

6. Clustering coefficient

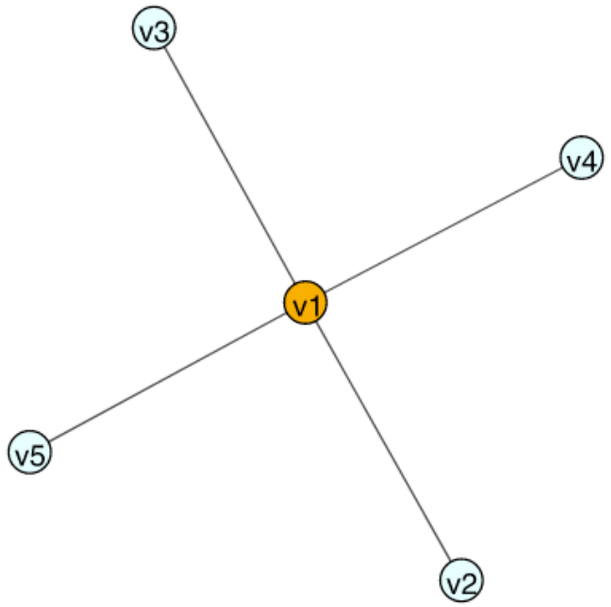
Gives the **fraction of possible interconnections** for neighbours of node i

If node v_1 is connected with v_2 and v_3, it is very likely that v_2 and v_3 are also connected.

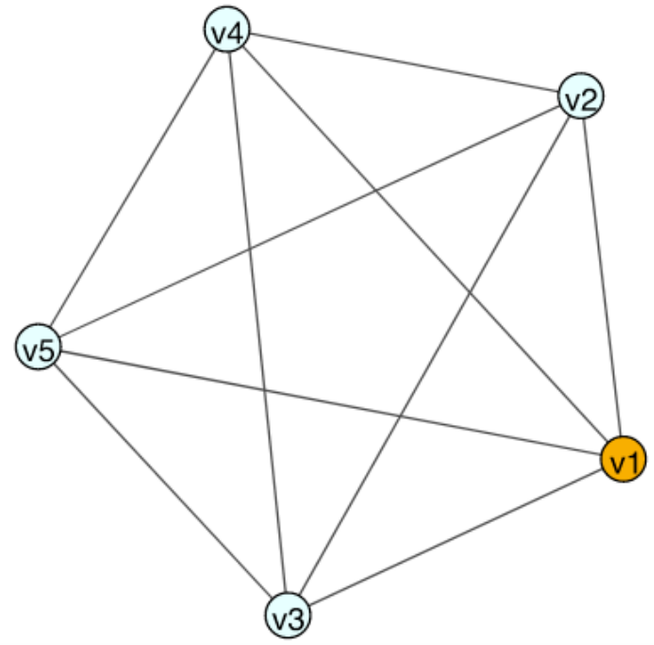
Takes into account degree of a node and the degree of its 1st neighbours



6. Clustering coefficient



$$0 \leq C_i \leq 1$$



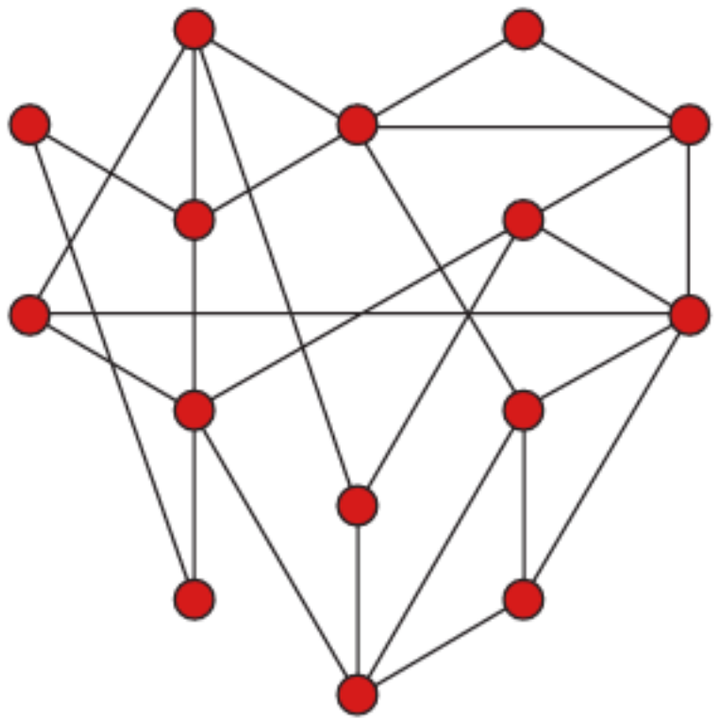
The global clustering coefficient $C(G)$ is simply the average of its clustering coefficients

7. Degree and clustering coefficient distribution

P(k) gives the probability that a selected node has exactly *k* edges

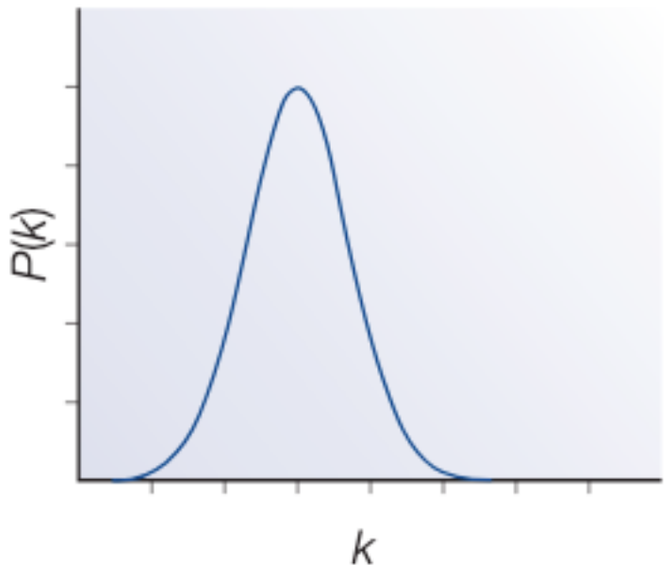
Allows distinguishing different kinds of networks

Random network



Poisson degree distribution

shows no highly connected nodes

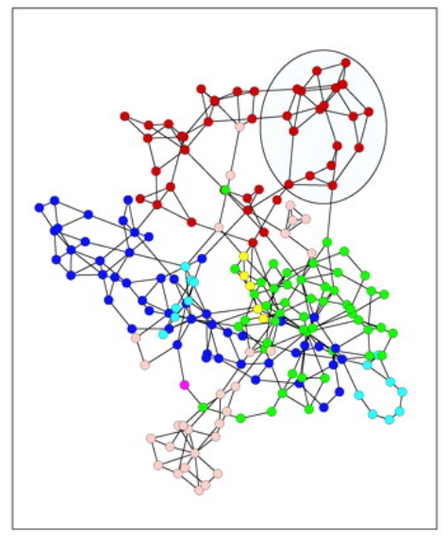


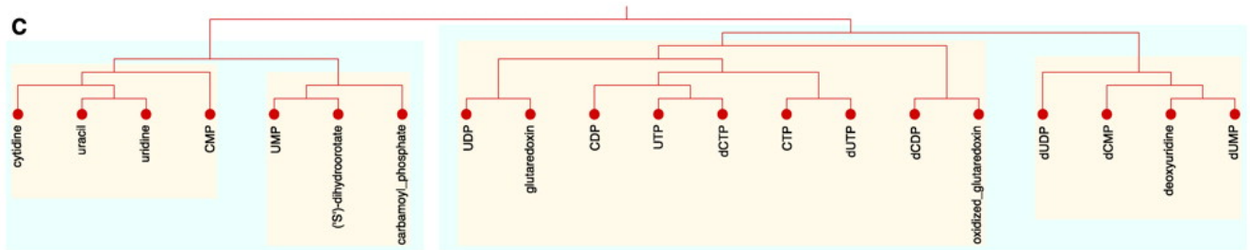
Most nodes have near <*k*>

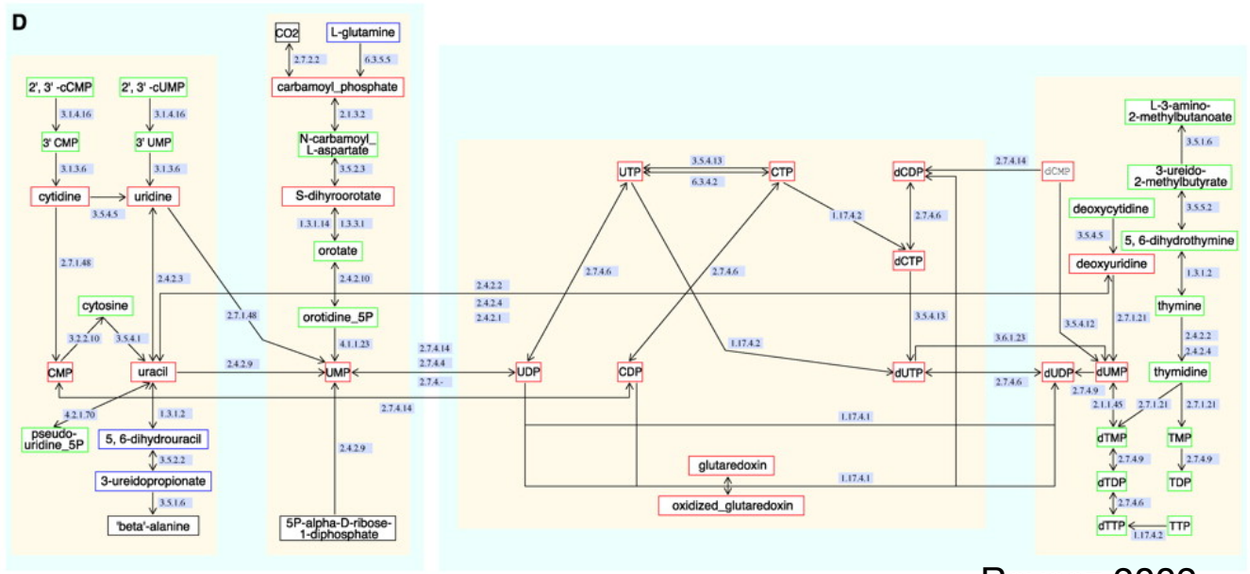
Metabolic networks show hierarchical topology

Metabolic networks of 43 organisms are organised into **small, tightly connected modules**

Their combination shows a hierarchical structure







Ravasz 2002

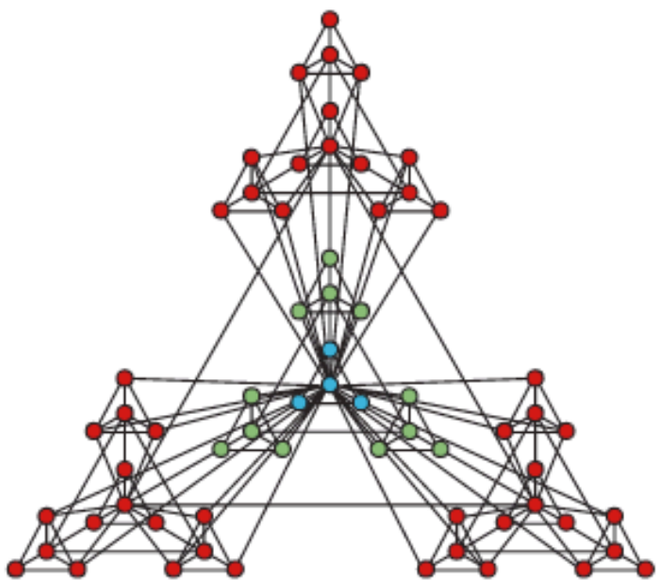
7. Degree distribution

Biological networks do not follow topology features of random networks.

Degree distribution *follows* the power-law $P(k) \propto k^{-\gamma}$

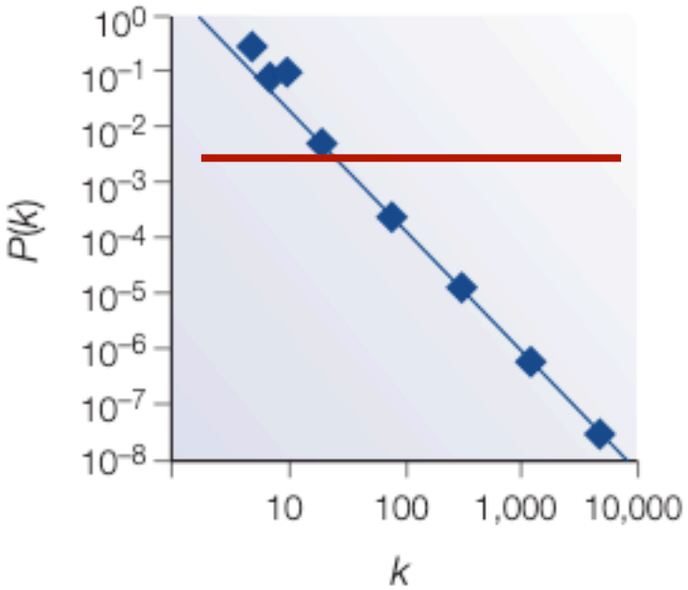
This allows for high robustness to node failure: removal of <80% nodes still retains paths between any two nodes

Hierarchical network

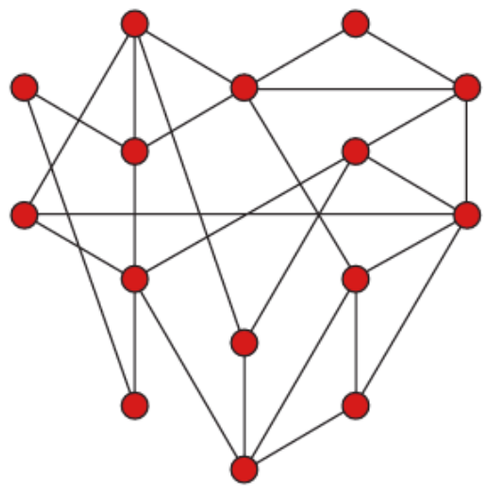


Degree distribution

shows many with low degrees
a few highly connected nodes



Random network



Barabasi 2004
Jeong 2000

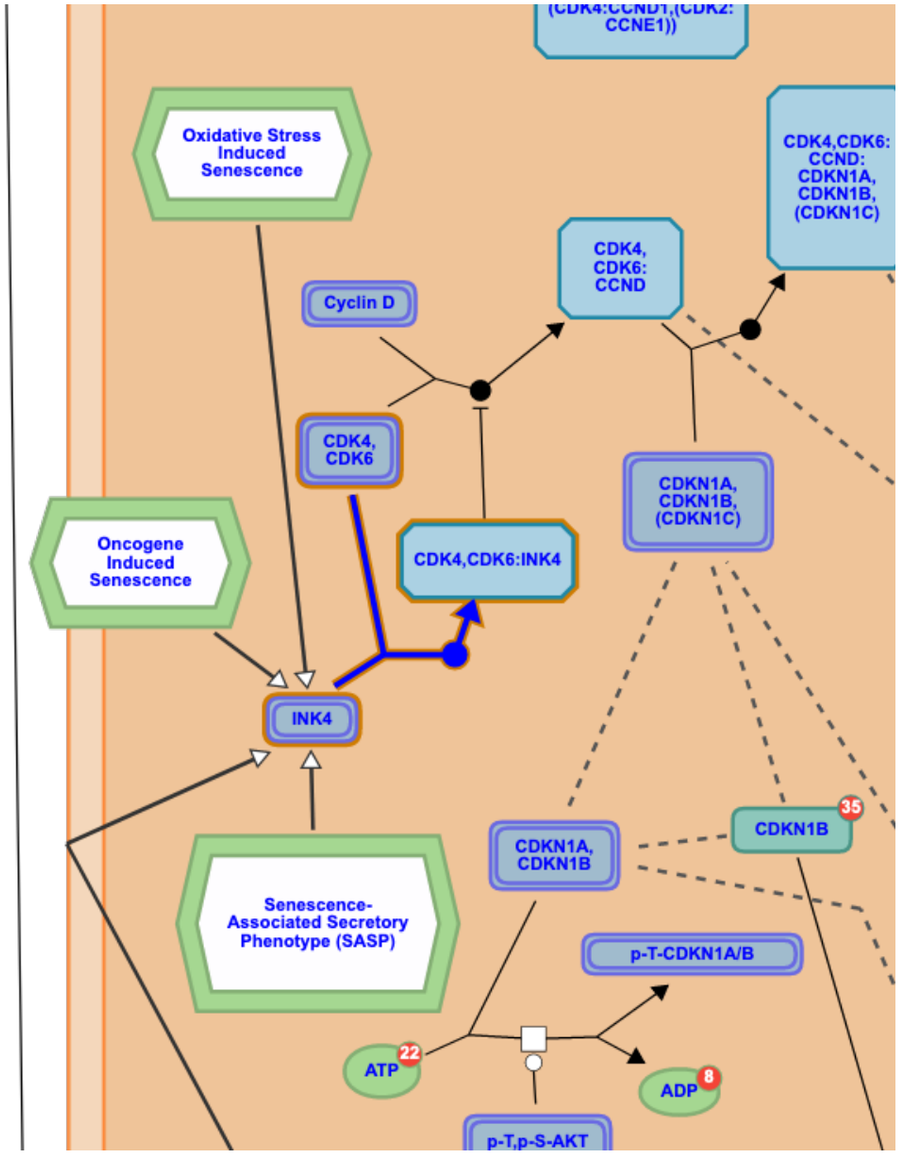
Overview

1. Introduction to network analysis
2. Terminology
3. Network inference
4. Key network properties
5. Community analysis
6. Workshop

What are modules?

Modules are physically or functionally associated nodes that work together to achieve a distinct function

Protein complexes are physical modules



What are modules?

In addition to physical or functional modules, one may identify other types of modules

Topological: derived from their high within-module degree

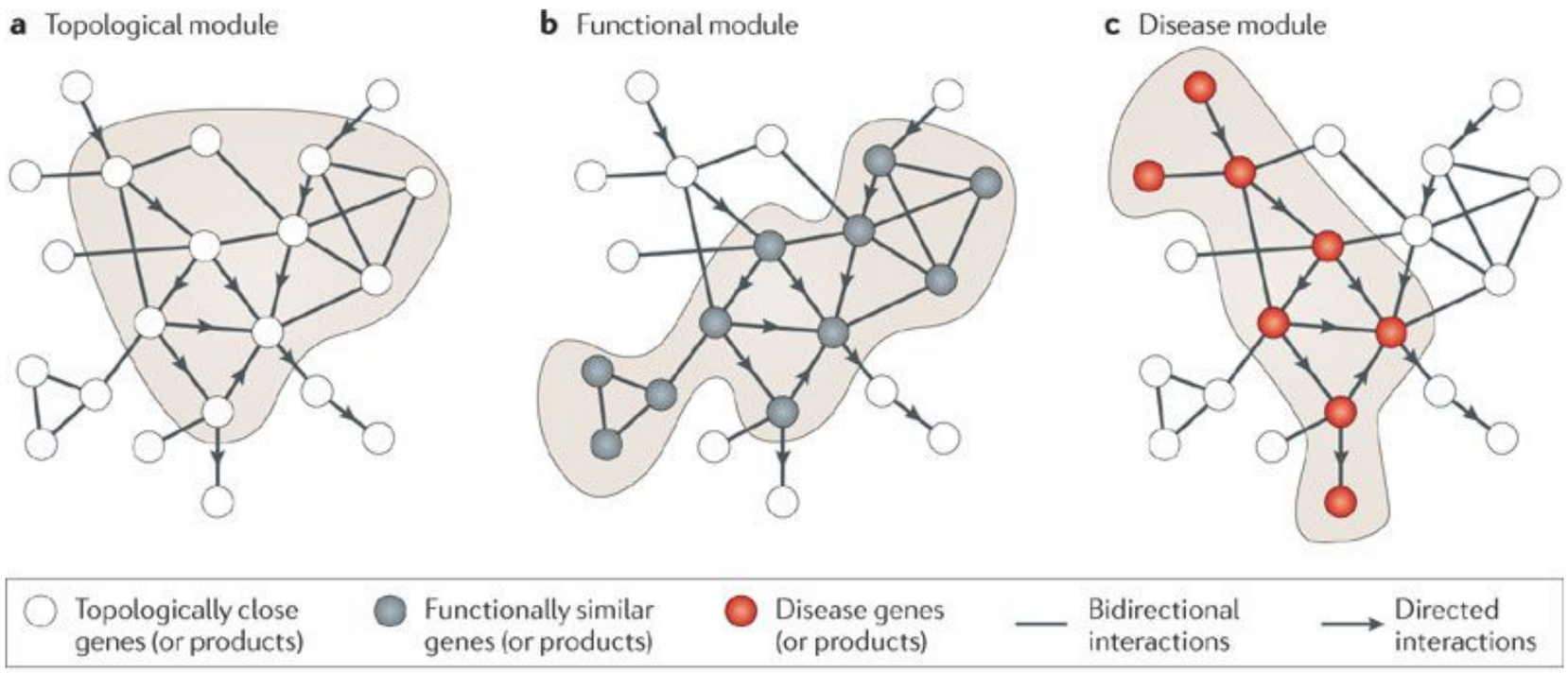
Disease: highly interconnected nodes associated with a disease response

Drug: highly interconnected nodes associated with a drug response

Subgroup: highly interconnected nodes associated with a sample subgroup (e.g. cancer subtype)

Tissue-, cell-type-specific: highly interconnected nodes associated with a specific tissue or cell type

Highly interlinked local regions of a network



Barabasi 2011

Modularity

Modularity is a property of the network

Modularity (Q) measures the tendency of a graph to be organised into modules

Modules computed by comparing probability that an edge is in a module vs what would be expected in a random network

$$Q \propto \sum_{s \in S} [(e_s) - (\text{expected } e_s)]$$

edges in group s

Random network with
same number of nodes, edges and
degree per node

$-1 < Q < 1$

Q = 1: much higher number of edges than expected by chance

Q = -1: lower number of edges than expected by chance

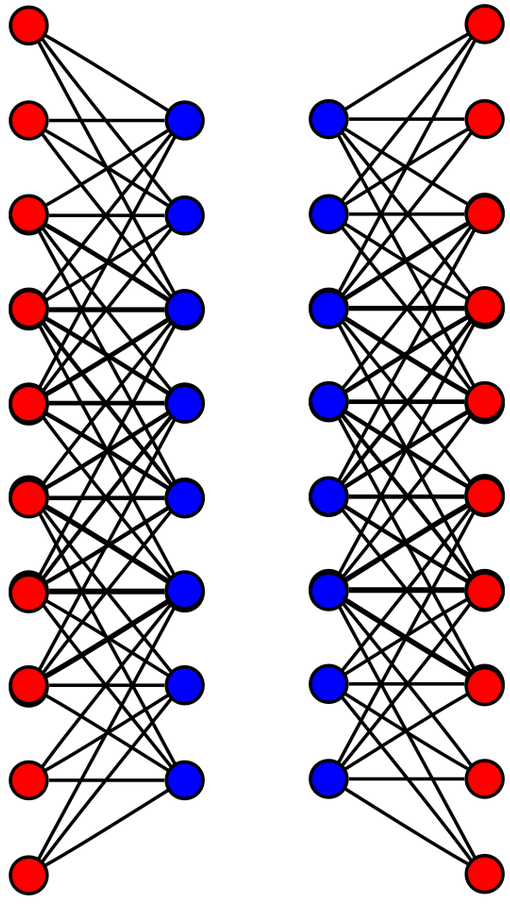
Q > 0.3 - 0.7 means significant community structure

Modularity

Modularity is different than **clustering coefficient**:

Take the following graph:

high Q but low connectivity (C)



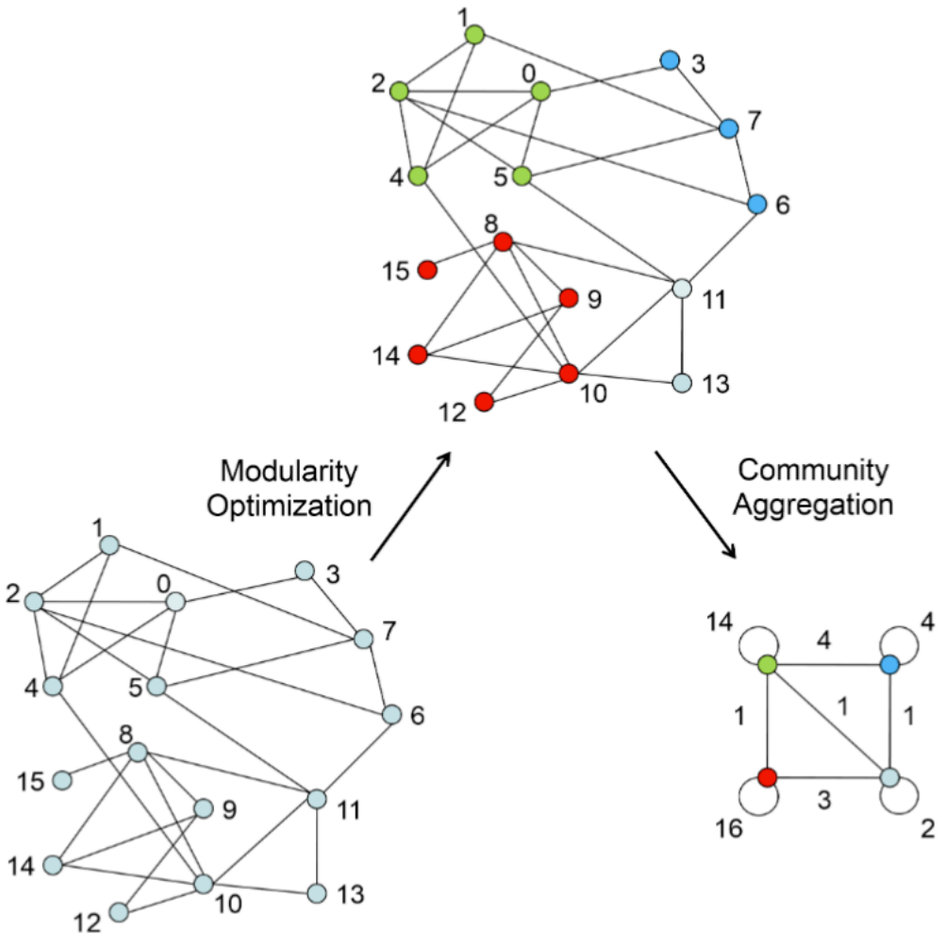
Module detection: Louvain algorithm

Phase 1: greedy modularity optimisation

1. Start with 1n/community
2. Compute Q by moving *i* to the community of *j*
3. If ΔQ>1, node is placed in community
4. Repeat 1-3 until no improvement is found. Ties solved arbitrarily

Phase 2: coarse grained community aggregation

5. Link nodes in a community into single node.
6. Self loops show intra-community associations
7. Inter-community weights kept
8. Repeat phase 1 on new network



Community characterisation

Clustering coefficient and degree distribution

Enrichment analysis

What do the nodes in each community have in common that could explain why they are in the same community?

Enrichment analysis

MSigDB

GSEA
Gene Set Enrichment Analysis

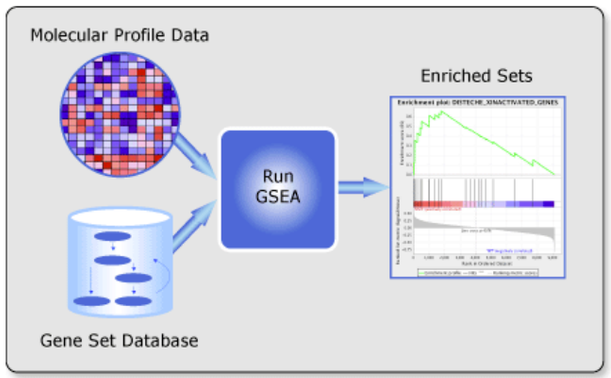
GSEA Home | Downloads | Molecular Signatures Database | Documentation | Contact

Overview

Gene Set Enrichment Analysis (GSEA) is a computational method that determines whether an a priori defined set of genes shows statistically significant, concordant differences between two biological states (e.g. phenotypes).

From this web site, you can:

- **Download** the GSEA software and additional resources to analyze, annotate and interpret enrichment results.
- **Explore the Molecular Signatures Database (MSigDB)**, a collection of annotated gene sets for use with GSEA software.
- **View documentation** describing GSEA and MSigDB.



What's New

20-Aug-2019: MSigDB 7.0 released. This is a major release that includes a complete overhaul of gene symbol annotations, Reactome and GO gene sets, and corrections to miscellaneous errors. See the release notes for more information.

20-Aug-2019: GSEA 4.0.0 released. This release includes support for MSigDB 7.0, plus major internal updates for Java 11 support and performance improvements. See the release notes for more information.

16-Jul-2018: MSigDB 6.2 released. This is a minor release that includes updates to gene set annotations, corrections to miscellaneous errors, and a handful of new gene sets. See the release notes for more information.

Follow @GSEA_MSigDB

License Terms

GSEA and MSigDB are available for use under these license terms.

Please register to download the GSEA software, access our web tools, and view the MSigDB gene sets. After registering, you can log in at any time using your email address. Registration is free. Its only purpose is to help us track usage for reports to our funding agencies.

Contributors

GSEA and MSigDB are maintained by the GSEA team. Our thanks to our many contributors. Funded by: National Cancer Institute, National Institutes of Health, National Institute of General Medical Sciences.

Citing GSEA

To cite your use of the GSEA software, please reference Subramanian, Tamayo, et al. (2005, PNAS 102, 15545-15550) and Mootha, Lindgren, et al. (2003, Nat Genet 34, 267-273).

Enrichr

Enrichr

Login | Register

21,153,478 lists analyzed
307,486 terms
154 libraries

Analyze | What's New? | Libraries | Find a Gene | About | Help

Gene-set Library	Terms	Gene Coverage	Genes per Term
Genes_Associated_with_NIH_Grants	32876	15886	9.0
Cancer_Cell_Line_Encyclopedia	967	15797	176.0
Achilles_fitness_decrease	216	4271	128.0
Achilles_fitness_increase	216	4320	129.0
Aging_Perturbations_from_GEO_down	286	16129	292.0
Aging_Perturbations_from_GEO_up	286	15309	308.0
Allen_Brain_Atlas_down	2192	13877	304.0
Allen_Brain_Atlas_up	2192	13121	305.0
ARCHS4_Cell-lines	125	23601	2395.0
ARCHS4_IDG_Coexp	352	20883	299.0
ARCHS4_Kinases_Coexp	498	19612	299.0
ARCHS4_TFs_Coexp	1724	25983	299.0
ARCHS4_Tissues	108	21809	2316.0
BioCarta_2013	249	1295	18.0
BioCarta_2015	239	1678	21.0
BioCarta_2016	237	1348	19.0
BioPlex_2017	3915	10271	22.0
ChEA_2013	353	47172	1370.0
ChEA_2015	395	48230	1429.0
ChEA_2016	645	49238	1550.0
Chromosome_Location	386	32740	85.0
Chromosome_Location_hg19	36	27360	802.0
CORUM	1658	2741	5.0
Data_Acquisition_Method_Most_Popular_Genes	12	1073	100.0
dbGaP	345	5613	36.0
DepMap_WG_CRISPR_Screens_Broad_CellLines_2019	558	7744	363.0
DepMap_WG_CRISPR_Screens_Sanger_CellLines_2019	325	6204	387.0
Disease_Perturbations_from_GEO_down	839	23939	293.0
Disease_Perturbations_from_GEO_up	839	23561	307.0
Disease_Signatures_from_GEO_down_2014	142	15406	300.0

Enrichment analysis

Important databases with gene-sets:

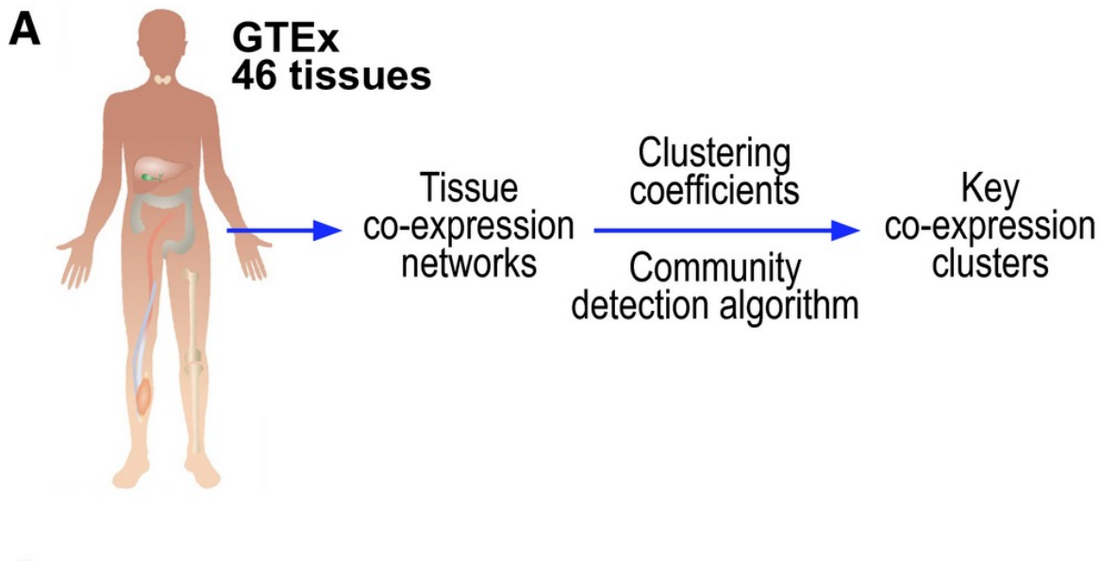
- MSigDB (gene)
- Enrichr (gene)
- KEGG (metabolite, gene)
- DIANA (miRNA)
- MetaboAnalyst (metabolite)
- DAVID (web)
- Reactome (web)

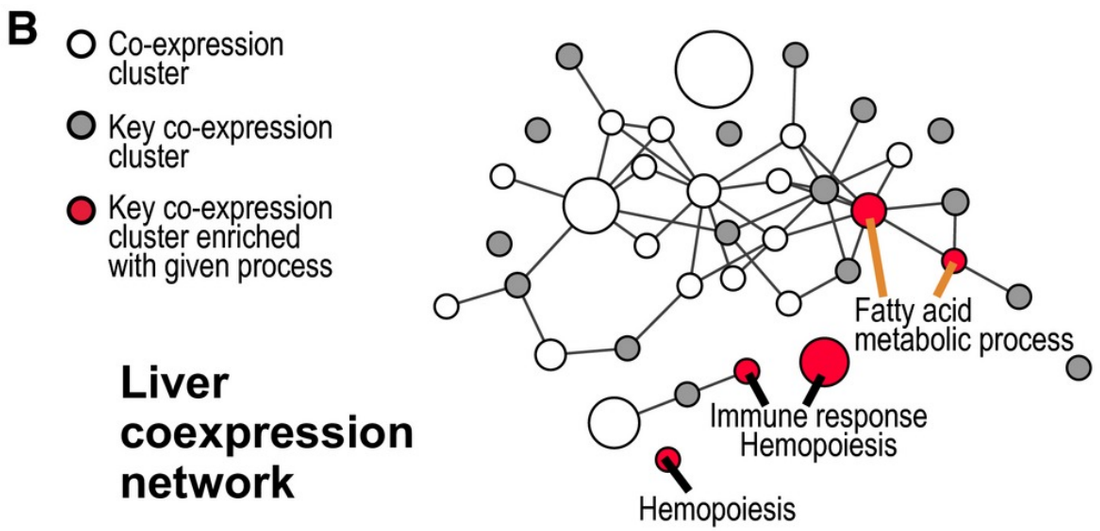
Creating custom sets and joint sets

Mapping your data to common IDs

- Easy for genes and proteins: use DAVID, Biomart, or MyGene (in Python or R)
- Harder for other feature types

Outcome

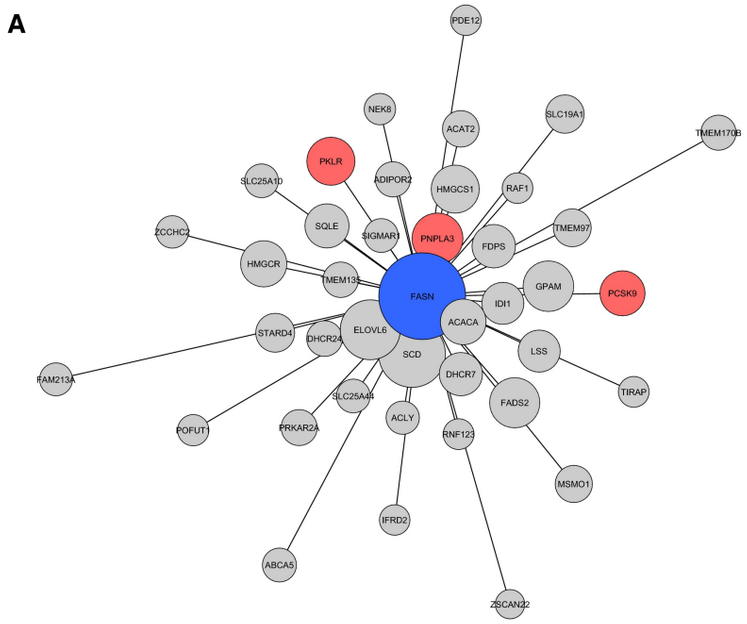




GO BPs from MSigDB

Network analyses identifies key stratifying genes

40-NN of FASN



Workshop objective:

Build and analyze a gene-metabolite association network

Additional reading

- Network Science - A fascinating textbook on graph theory and network analysis.
- Communication dynamics in complex brain networks - Interesting discussion about whether and how network topology may be applied to study the brain networks.
- A Systematic Evaluation of Methods for Tailoring Genome-Scale Metabolic Models - General review and discussion on methods to use in genome-scale metabolic models.
- Analysis of Biological Networks - General introduction into biological networks, network notation, and analysis, including graph theory.
- Multi-omics approaches to disease - Introduction to how integrative approaches may be applied in disease

Additional references displayed as hyperlinks in each slide.