香港中文大學
The Chinese University of Hong Kong

CU Medicine
香港中文大學醫學院
Faculty of Medicine
The Chinese University of Hong Kong

Network Analyses in the Microbiome

Joint Graduate Seminar
Supervisor: Prof. Margaret Ip
Co-Supervisor:Prof. Sishuo Wang
        Dr. Haokui Zhou
        Dr. Mingjing Luo
Year 1 PhD Student: Zheng Ruiqi
Date: 23rd Nov 2023
Department: Microbiology

overview

香 港 中 文 大 學
The Chinese University of Hong Kong

CU Medicine
香港中文大學醫學院
Faculty of Medicine
The Chinese University of Hong Kong
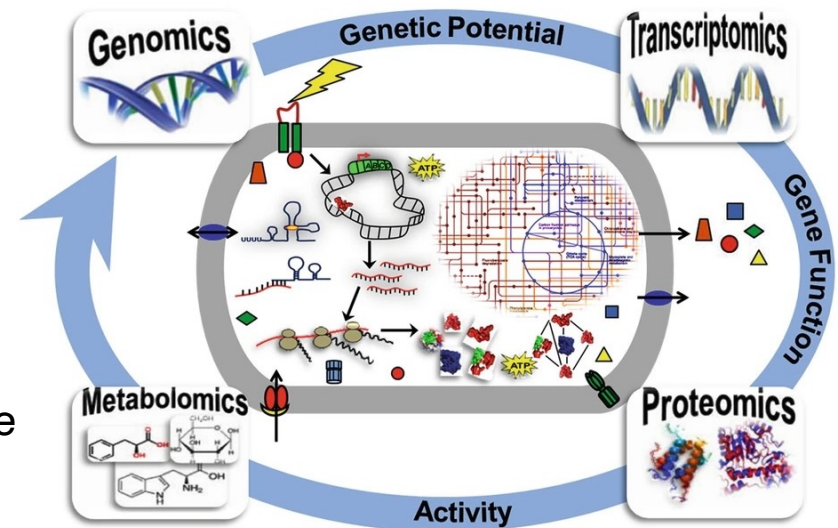
Microbiology meets network theory

Microbiomes are complex microbial communities. Interactions influence the structure and the function of such communities.

More than 99% of microorganisms in nature could not be cultured alone *in vitro*!

High-throughput Omics technologies:
- high volume of data generation
- high quality of data interpretation
- fairly acceptable cost

Effective computational analysis techniques are necessary!

Dey, D., et al. Integrating Omics Technologies to Understand Microbial Systems. 2021

香港中文大學
The Chinese University of Hong Kong

CU Medicine 香港中文大學醫學院
Faculty of Medicine
The Chinese University of Hong Kong

Microbiology meets network theory

What can we do with a Network?

Obtain info that we wouldn't get by analysing individual components!

- Important protein calls HUBS (have a lot of connections-degree)
- Community of proteins (belongs to the same pathway or Complex)
- Simulate in silico the bacteria behaviour
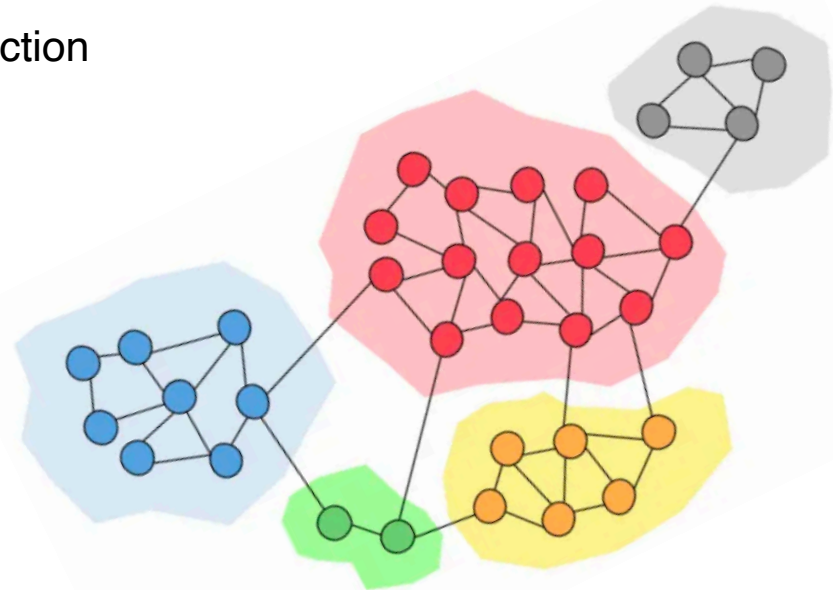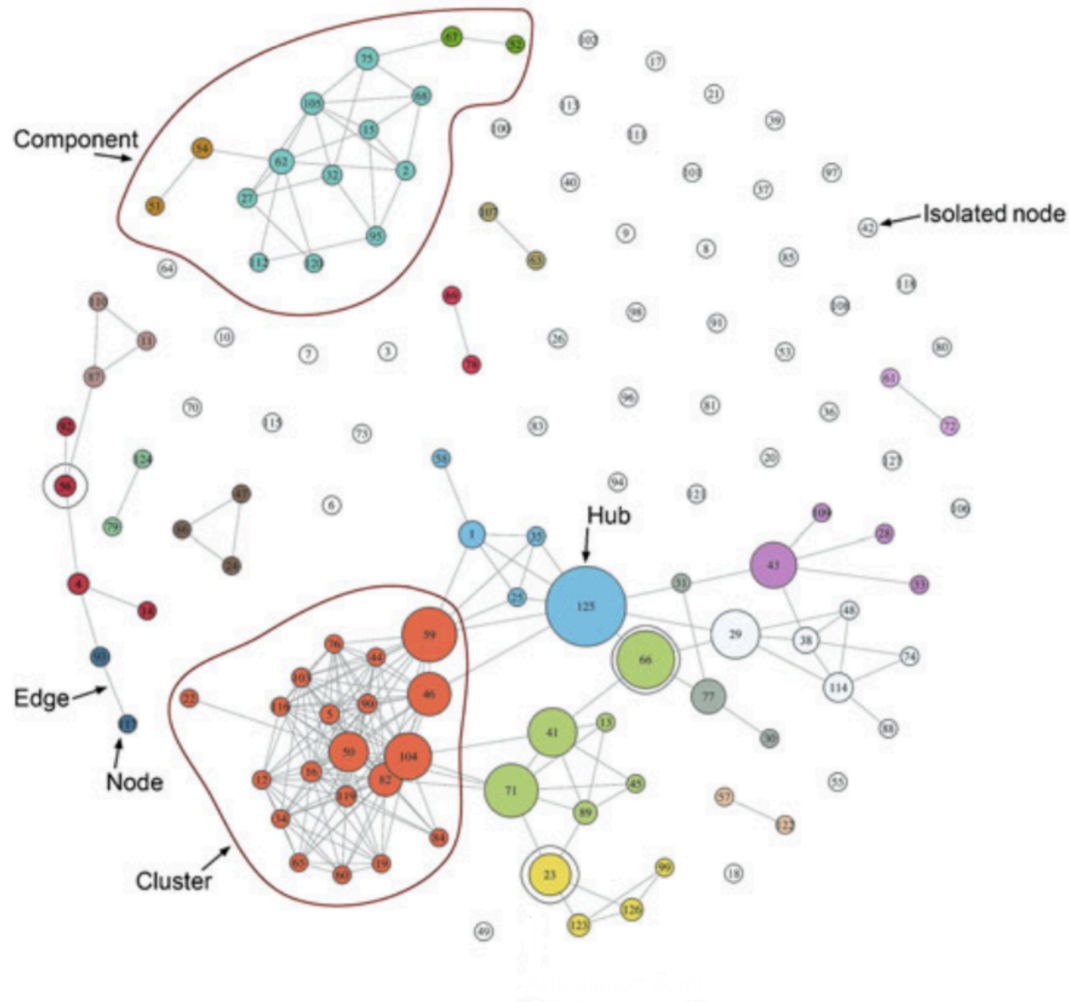- Formulate new hypothesis about function

Communities

Image by Thamindu Dilshan Jayawickrama

香港中文大學
The Chinese University of Hong Kong

CU Medicine
香港中文大學醫學院
Faculty of Medicine
The Chinese University of Hong Kong

3

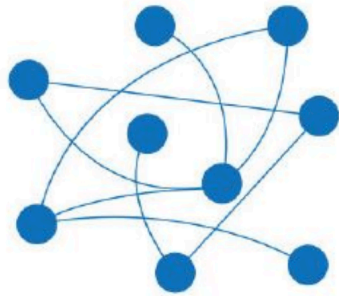# Basic network theory concepts

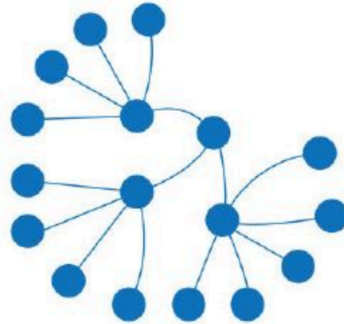
Node
Edge
Hub
Component
Module
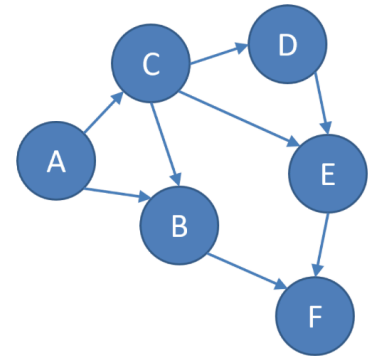Degree

Image by Jean-Claude Walser

Basic network theory concepts

Random network
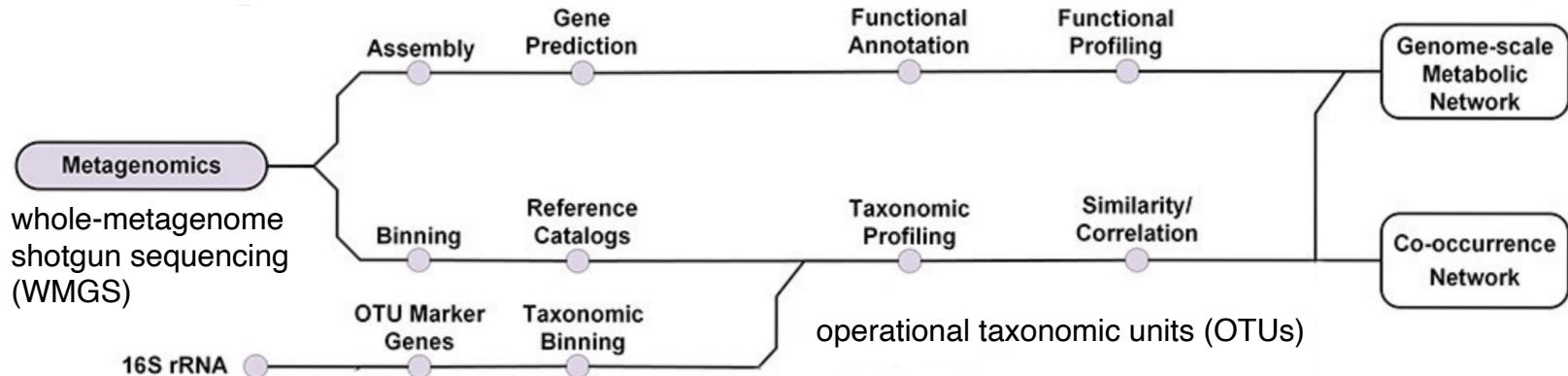
Scale-free network

Bayesian network

Scale-free network: most of nodes have only a few connections to other nodes, whereas some hubs are connected to many other nodes in the network.Real-world networks are often claimed to be scale free.

Bayesian network: a probabilistic graphical model that represents a set of variables and their conditional dependencies via a directed acyclic graph (DAG).

Networks from metagenomics

Network modelling methods:

whole-metagenome shotgun sequencing (WMGS)

operational taxonomic units (OTUs)

Tools: UPARSE, MetaPhlan4, mOTUs2, QIIME2, HUMAnN3

Constructing Networks

Dissimilarity-Based Methods
Correlation-Based Methods
Regression-Based Methods
Probabilistic Graphical Models

香港中文大學
The Chinese University of Hong Kong

CU Medicine
香港中文大學醫學院
Faculty of Medicine
The Chinese University of Hong Kong
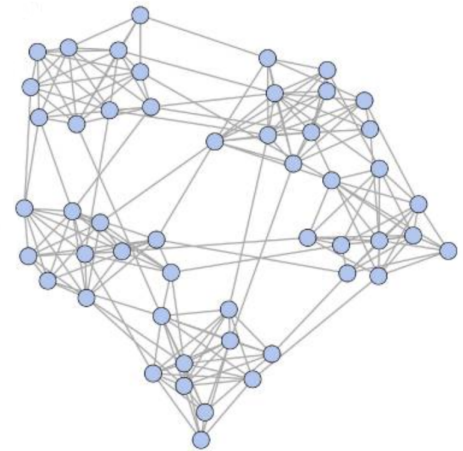
Networks from metagenomics

Detecting Hub node from Networks

important nodes or hubs:
most influential member in the microbial community
most essential microbe for community stability
organism responsible for disease transmission

Detecting Biologically Important Clusters from Networks

Clusters/modules: provides information about the local
interaction patterns in the network and their contribution to the
overall structure, connectivity, and function of the network
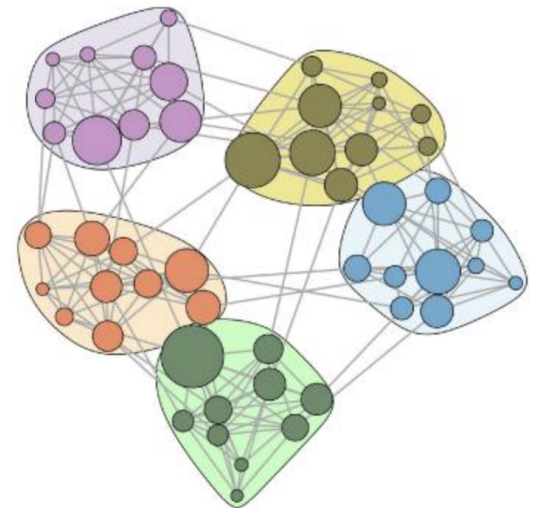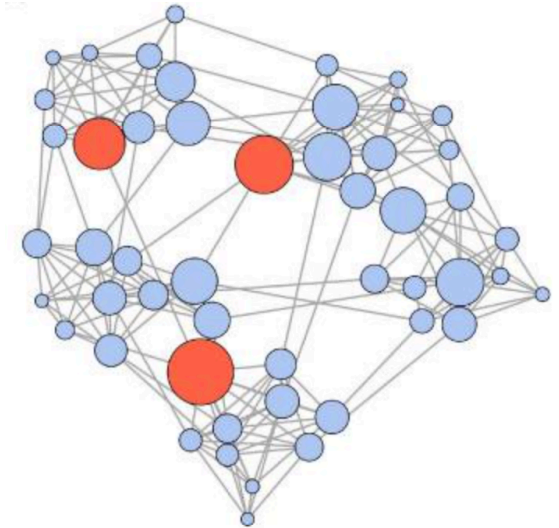
Networks from metagenomics

Networks at the taxonomic level

Co-occurrence networks

151 soil samples

⬇ metagenome data analyses

2798 OTUs

⬇ correlation analysis

Co-occurrence networks
(296 nodes (OTUs) and 679 edges)

⬇ subsequent topological analysis

Modules

⬇

OTUs colored by taxonomy
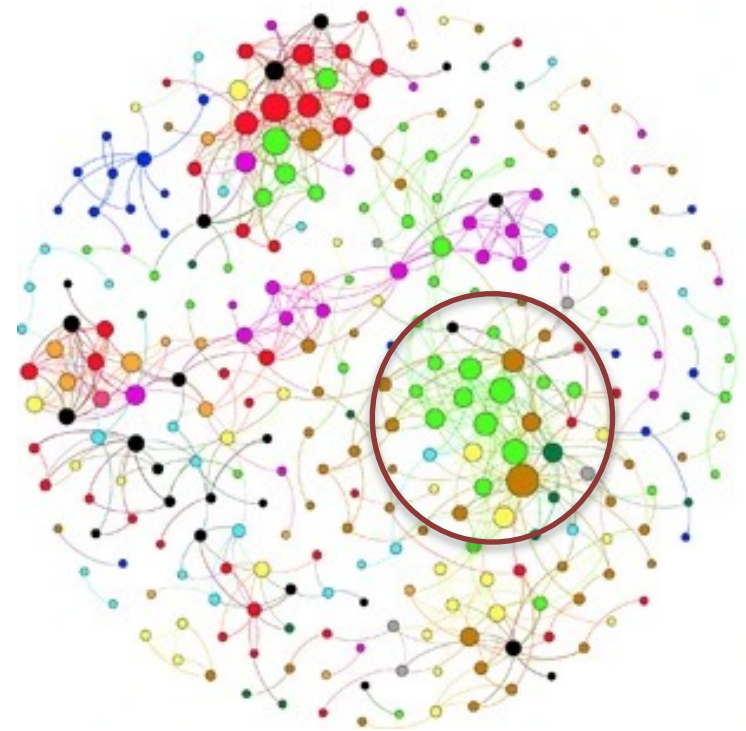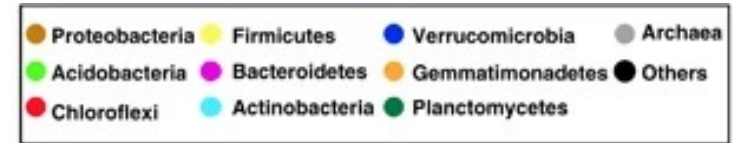
co-occurrence networks in soil
microbial communities

Albert Barberán, et al.ISME J. 2012 Feb; 6(2): 343–351.

Networks from metagenomics

Networks at the functional level

50 samples from 10 typical environments

260 ARG subtypes belonging to 18 ARG types taxonomic abundance

Significant Spearman's rank correlation between the microbial diversity and the ARGs diversity

Co-occurrence network

Nodes were coloured according to ARG types and genus
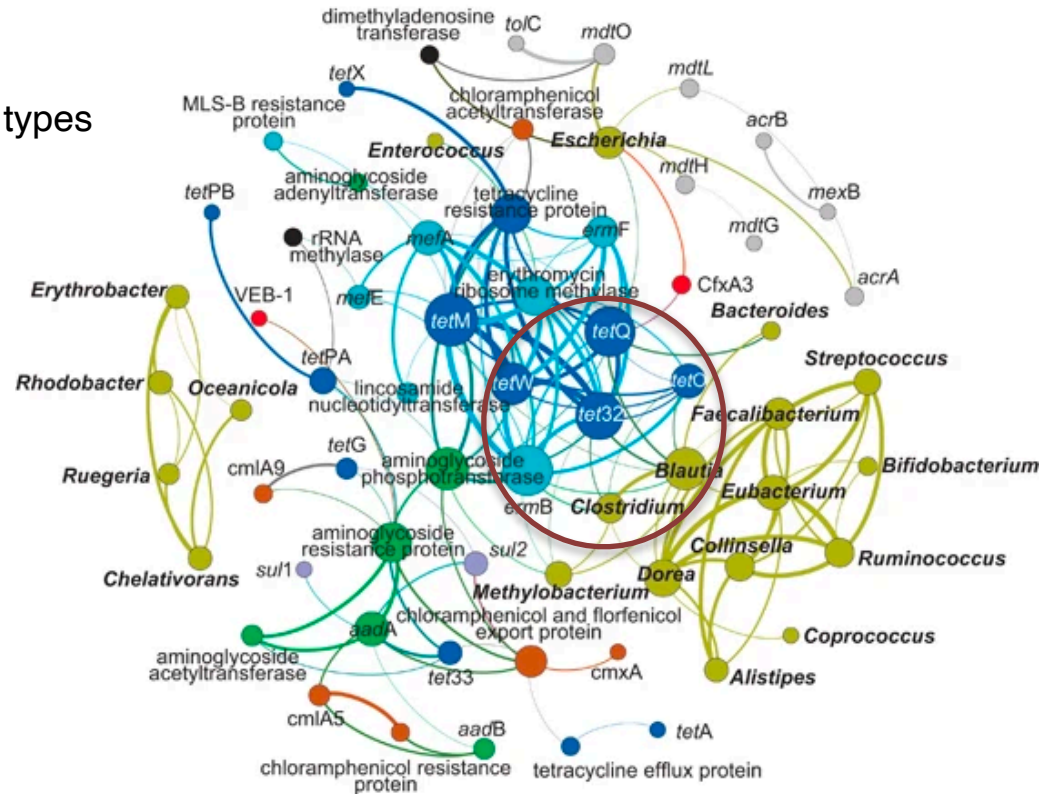
The Network analysis revealing the co-occurrence patterns between antibiotic resistance genes (ARGs) subtypes and microbial taxa in multiple environments.

香港中文大學
The Chinese University of Hong Kong

CU Medicine
香港中文大學醫學院
Faculty of Medicine
The Chinese University of Hong Kong

Networks from metatranscriptomics

Network modelling methods:
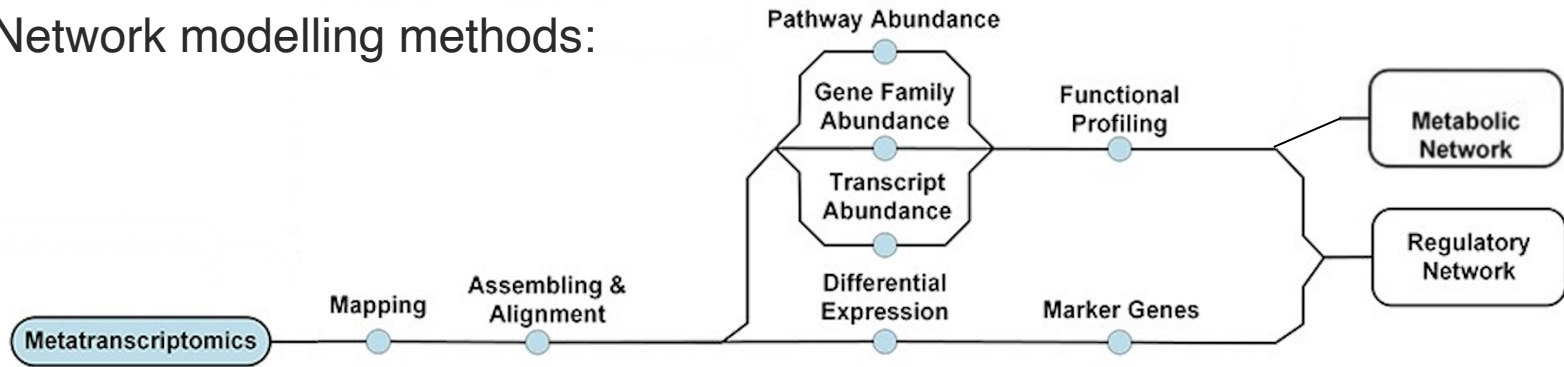
Tools: MetaTrans, SAMSA

More pertinent information on functional activity: metatranscriptomics can reveal details of genes that are transcriptionally active under specific conditions and time

香港中文大學
The Chinese University of Hong Kong

CU Medicine 香港中文大學醫學院
Faculty of Medicine
The Chinese University of Hong Kong

Networks from metatranscriptomics
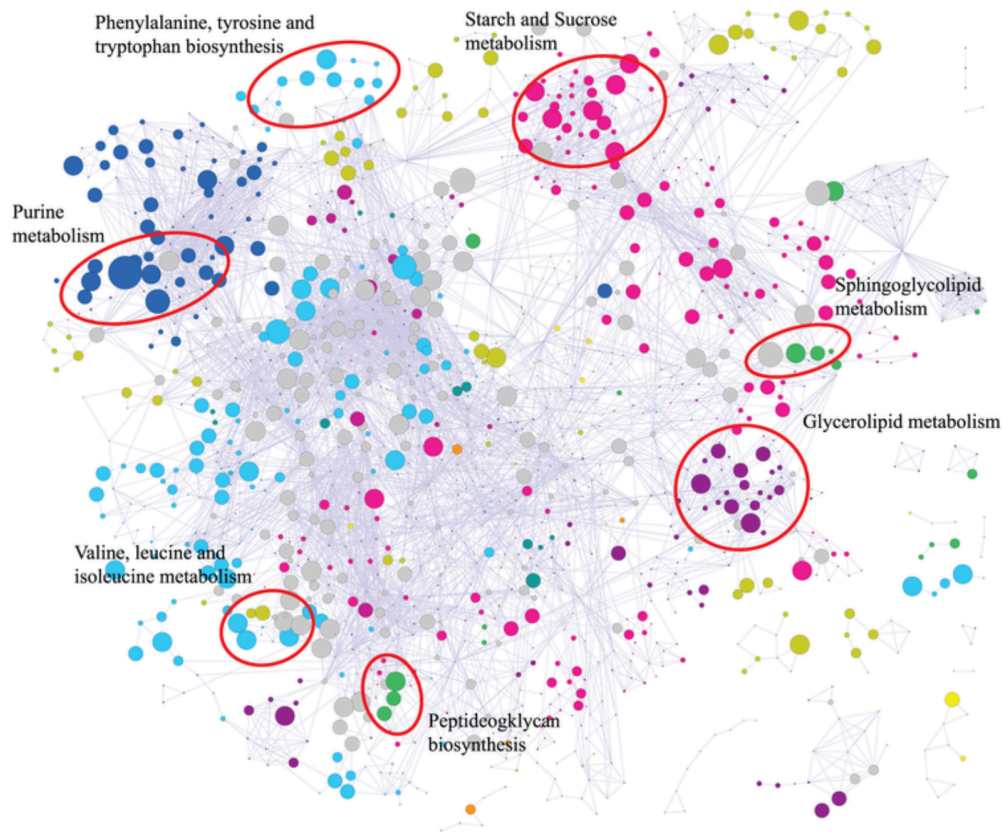
12 cecal and colon derived samples from mice

↓

236,769 unique transcripts

↓

relative expression of each gene family

↓

Metabolic networks

↓

Colour of node indicates functional category of enzyme as defined by KEGG superclasses

Metatranscriptome data mapped in the context of a global metabolic network

Galeb S, et al.Nature Microbiology volume 3, pages 356–366 (2018)

香港中文大學
The Chinese University of Hong Kong

CU Medicine
香港中文大學醫學院
Faculty of Medicine
The Chinese University of Hong Kong

Networks from metaproteomics

Network modelling methods:
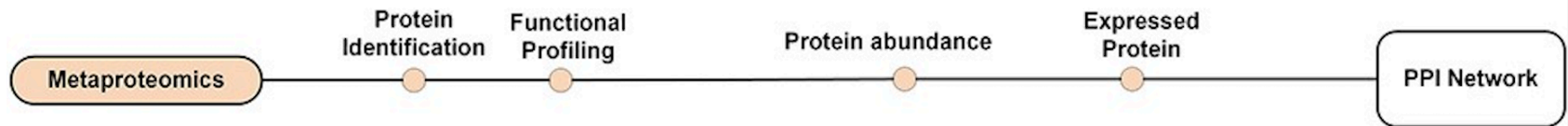
Tools: IdentiPy, Trans-Proteomic Pipeline, compleXView

Protein–protein interaction (PPI) networks describe physical and selective contacts that happen between pairs of proteins, in certain molecular regions and in a defined biological context.

Networks from metaproteomics
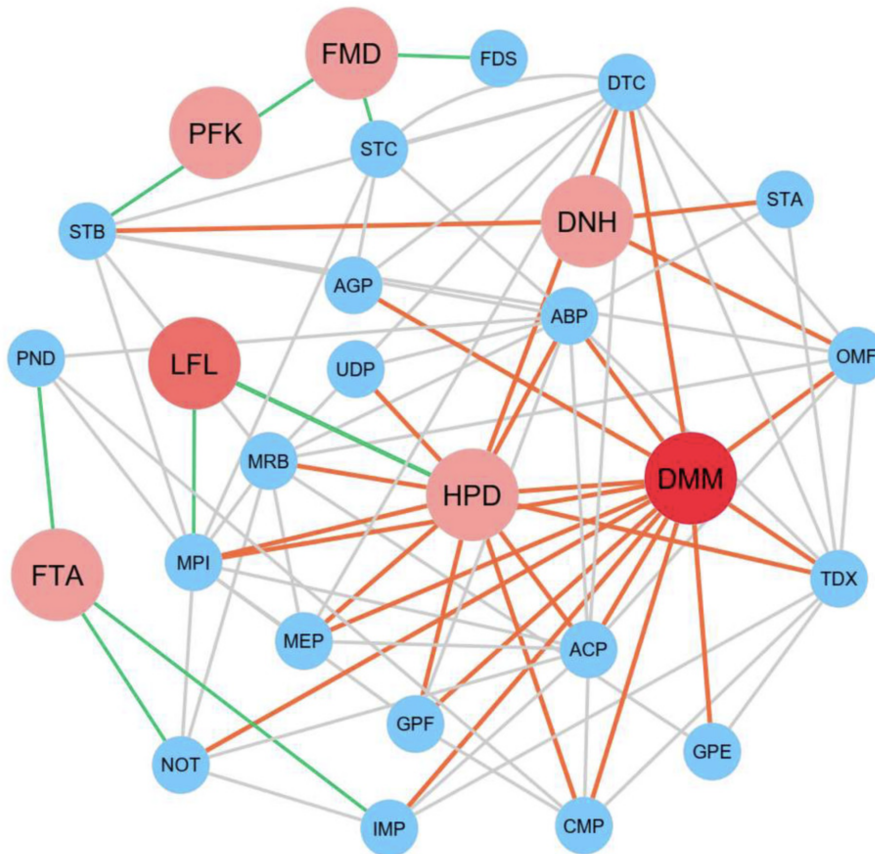
18 infants: 11 healthy controls and 7 patients with atopic dermatitis

18 fecal samples

49,973 annotated proteins out of 68,232 total proteins

PPI network

Reporter protein network involving ubiquinone and other quinone biosynthesis as well as energy supply.

香港中文大學
The Chinese University of Hong Kong

CU Medicine 香港中文大學醫學院
Faculty of Medicine
The Chinese University of Hong Kong

Networks from metabolomics

Network modelling methods:
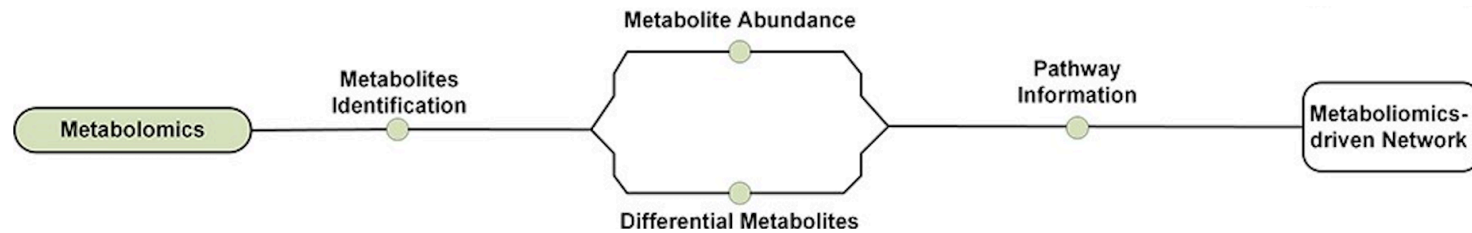
Tools: Pathos, MetaboAnalyst, Netome

Metabolomics-driven networks: consisting of nodes as metabolites and edges as metabolic reactions, provide a comprehensive description of a community's metabolic processes.

香港中文大學
The Chinese University of Hong Kong

CU Medicine HONG KONG
香港中文大學醫學院
Faculty of Medicine
The Chinese University of Hong Kong

Networks from metabolomics
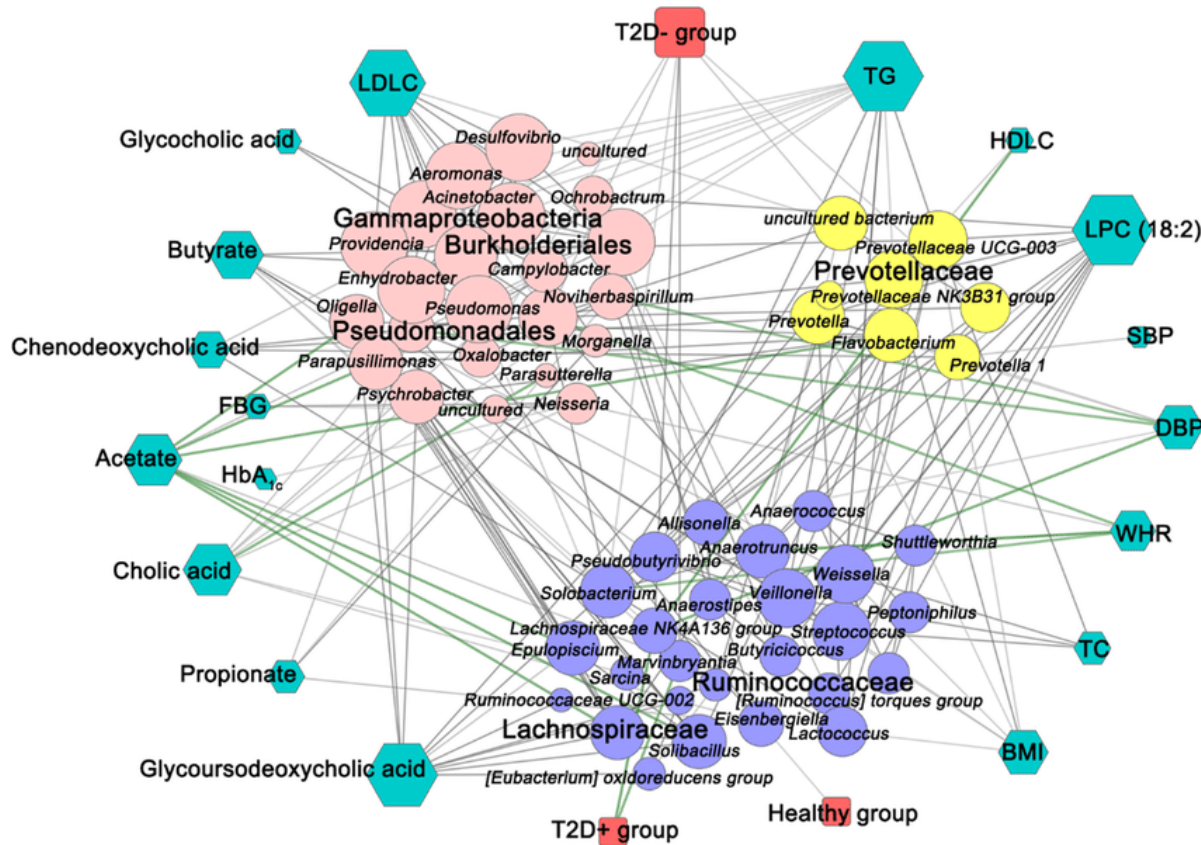
65 T2D patients:
49 with and 16 without
diabetic complications,
and 35 healthy controls

The correlation network between gut microbiota and metabolic traits in T2D and healthy groups

Lijuan Zhao, et al. Endocrine. 2019 Dec;66(3):526-537.

香港中文大學
The Chinese University of Hong Kong

CU Medicine
香港中文大學醫學院
Faculty of Medicine
The Chinese University of Hong Kong

Networks from multi-omics

Each omics analysis has its limitation

1. Integrated metagenomic, metatranscriptomic and metaproteomic data to construct a community-wide metabolic network

2. Apply Bayesian networks, to elucidate the underlying molecular mechanisms of diseases by associating the microbial community, both at the level of taxonomy and functionality, with disease phenotypes and clinical measurements
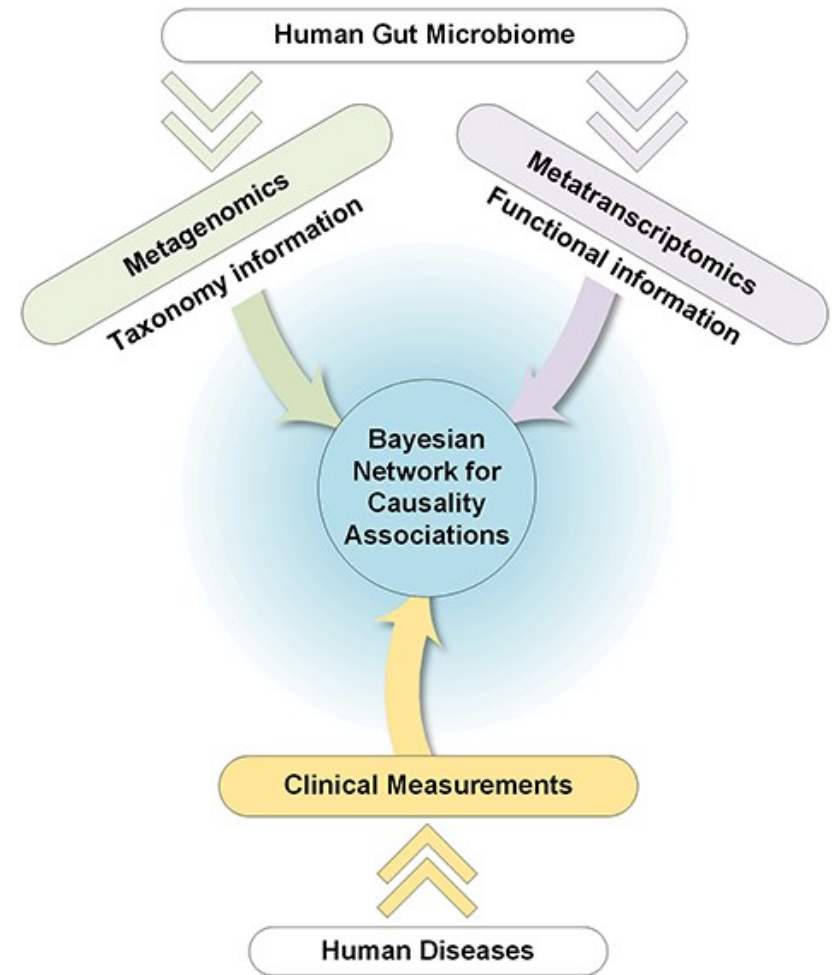
香港中文大學
The Chinese University of Hong Kong

CU Medicine 香港中文大學醫學院
Faculty of Medicine
The Chinese University of Hong Kong

Networks from multi-omics

132 individuals over a year
profiled every 2 weeks, on average, for
different omic types
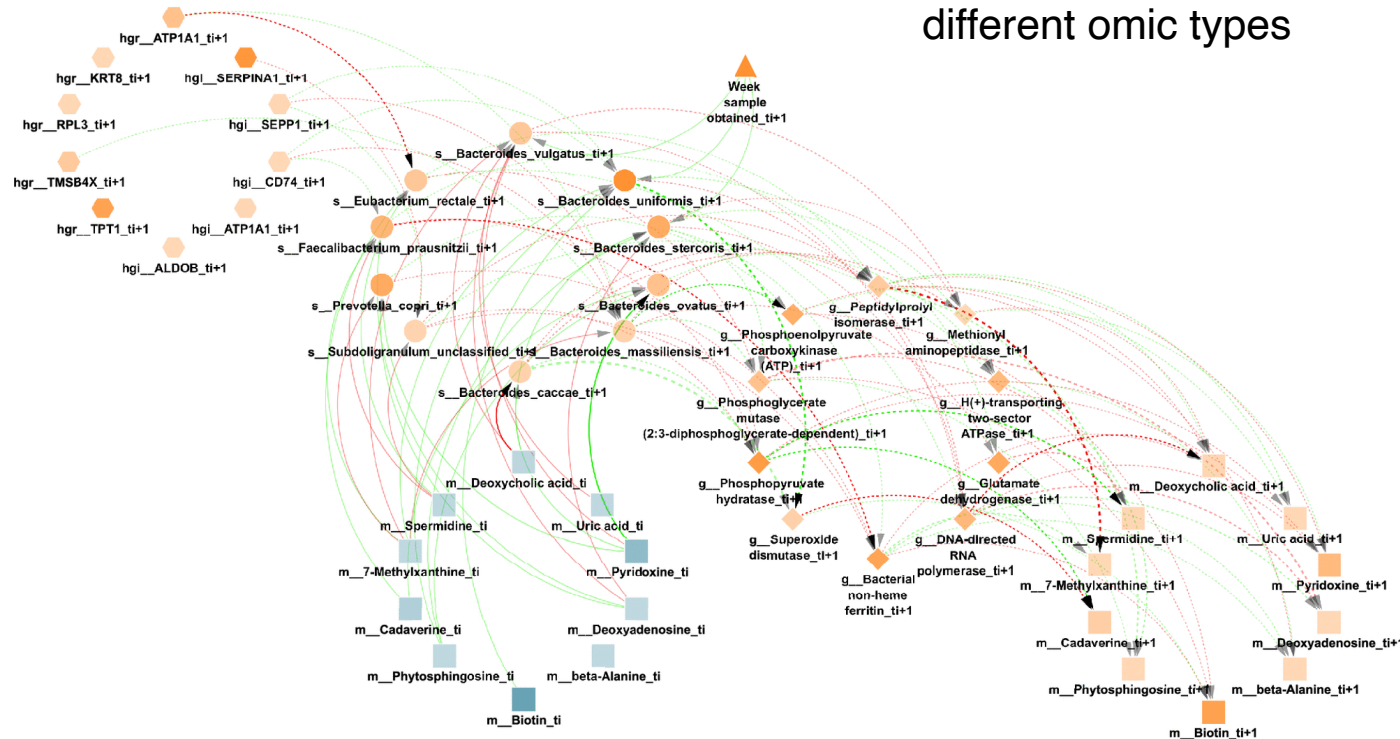
Dynamic Bayesian network (DBN): a Bayesian network (BN) which relates variables to each other over adjacent time steps.

香港中文大學
The Chinese University of Hong Kong

CU Medicine
香港中文大學醫學院
Faculty of Medicine
The Chinese University of Hong Kong

Challenges

- What to do with rare taxa?

  The majority of taxa in sequencing data are only found in very few samples. This means that a large part of sequencing data consists of zeros.

- How to deal with environmental factors?

  It is difficult to determine whether an edge in a microbial network is due to a common response to an environmental factor (or a third taxon) or represents a direct interaction between two taxa.

- How to evaluate microbial network construction in silico?

  Evaluations are carried out to assess which tools infer the most accurate networks and to explore how sample number and other data properties affect tool performance.

- How well do microbial networks represent ecosystems?

  Assuming that network inference is sufficiently accurate, can network properties such as modularity, and network density give useful information about the ecosystem under study?

香港中文大學
The Chinese University of Hong Kong

CU Medicine 香港中文大學醫學院
Faculty of Medicine
The Chinese University of Hong Kong

Summary

- The microbiome is a complex system of microbes

- With the development of network theories and meta-omics data, network models are widely used to study microbial communities.

- Multi-omics data integration in networks provides a comprehensive view of microbial communities and their interactions with other factors.

- Challenges remain, including detecting rare microbes, incomplete genome annotation, and selecting appropriate network models.

References

Zhaoqian Liu, Anjun Ma, Ewy Mathé, Marlena Merling, Qin Ma, Bingqiang Liu, Network analyses in microbiome based on high-throughput multi-omics data, *Briefings in Bioinformatics*, Volume 22, Issue 2, March 2021

Kaiser, Thomas et al. "Network-based approaches for the investigation of microbial community structure and function using metagenomics-based data." *Future microbiology* vol. 17 (2022): 621-631.

Jiang, Duo et al. "Microbiome Multi-Omics Network Analysis: Statistical Considerations, Limitations, and Opportunities." *Frontiers in genetics* vol. 10 995. 8 Nov. 2019

Faust, Karoline. "Open challenges for microbial network construction and analysis." *The ISME journal* vol. 15,11 (2021): 3111-3118.

Dey, D., Mekala, L.P., Mohammed, M. (2021). Integrating Omics Technologies to Understand Microbial Systems. In: Kumar, A., Kumar, R., Shukla, P., Pandey, M.K. (eds) Omics Technologies for Sustainable Agriculture and Global Food Security Volume 1. Springer, Singapore.

Barberán, Albert et al. "Using network analysis to explore co-occurrence patterns in soil microbial communities." *The ISME journal* vol. 6,2 (2012): 343-51.

Li, Bing et al. "Metagenomic and network analysis reveal wide distribution and co-occurrence of environmental antibiotic resistance genes." *The ISME journal* vol. 9,11 (2015): 2490-502. doi:10.1038/ismej.2015.59

香港中文大學
The Chinese University of Hong Kong

CU Medicine
香港中文大學醫學院
Faculty of Medicine
The Chinese University of Hong Kong

20

References

Xiong, Xuejian et al. "Generation and analysis of a mouse intestinal metatranscriptome through Illumina based RNA-sequencing." *PloS one* vol. 7,4 (2012): e36009.

Kingkaw, Amornthep et al. "Analysis of the infant gut microbiome reveals metabolic functional roles associated with healthy infants and infants with atopic dermatitis using metaproteomics." *PeerJ* vol. 8 e9988. 25 Sep. 2020, doi:10.7717/peerj.9988

Zhao, Lijuan et al. "Comprehensive relationships between gut microbiome and faecal metabolome in individuals with type 2 diabetes and its complications." *Endocrine* vol. 66,3 (2019): 526-537.

Ruiz-Perez, Daniel et al. "Dynamic Bayesian Networks for Integrating Multi-omics Time Series Microbiome Data." *mSystems* vol. 6,2 e01105-20. 30 Mar. 2021

Layeghifard M, Hwang DM, Guttman DS. Disentangling Interactions in the Microbiome: A Network Perspective. Trends Microbiol. 2017 Mar;25(3):217-228. doi: 10.1016/j.tim.2016.11.008. Epub 2016 Dec 2. PMID: 27916383; PMCID: PMC7172547.

香港中文大學
The Chinese University of Hong Kong

CU Medicine
香港中文大學醫學院
Faculty of Medicine
The Chinese University of Hong Kong

21

Thank you!