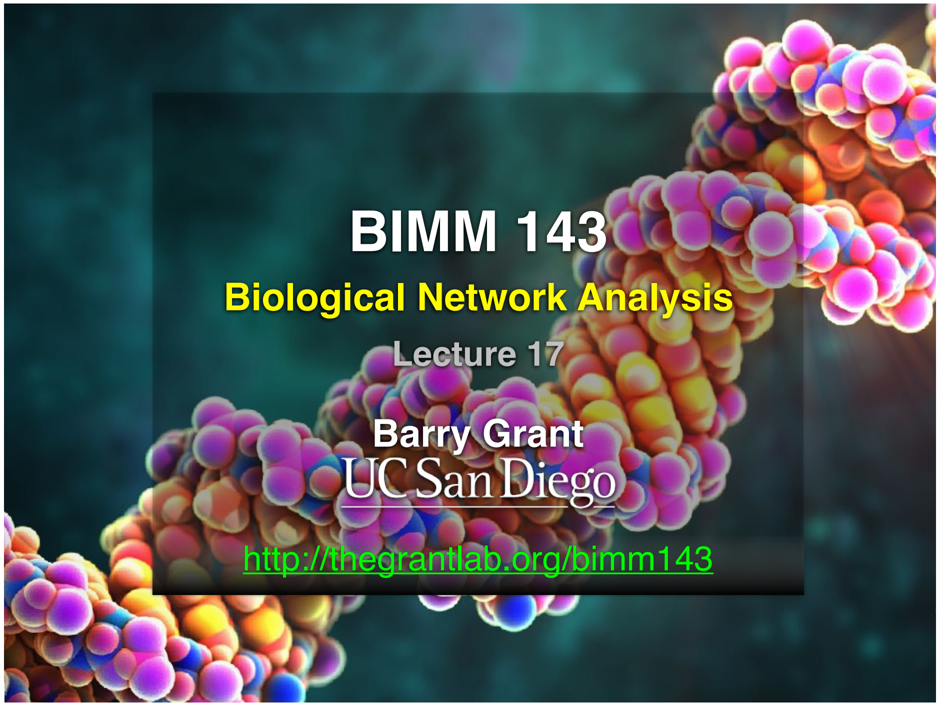


BIMM 143

Biological Network Analysis

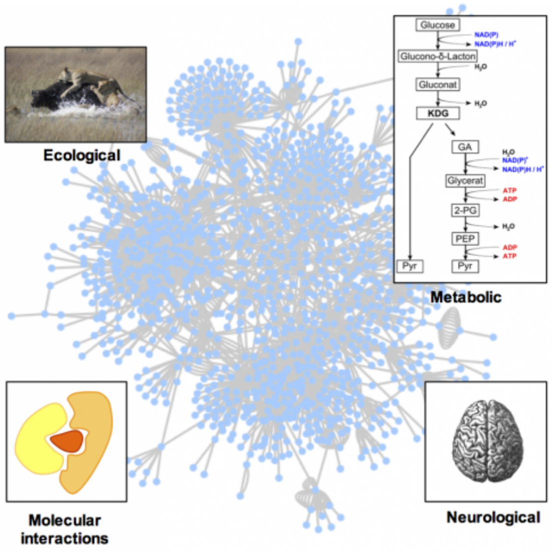
Lecture 17

Barry Grant

UC San Diego

http://thegrantlab.org/bimm143

Networks can be used to model many types of biological data



TODAYS MENU:

- Network introduction
- Network visualization
- Network analysis
- Hands-on:

  Cytoscape and R (igraph) software tools for network visualization and analysis

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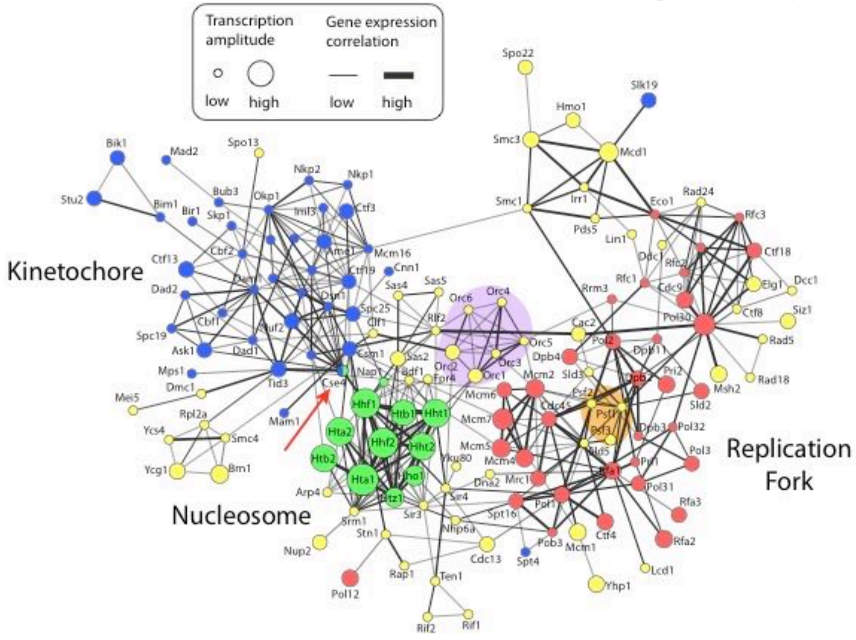
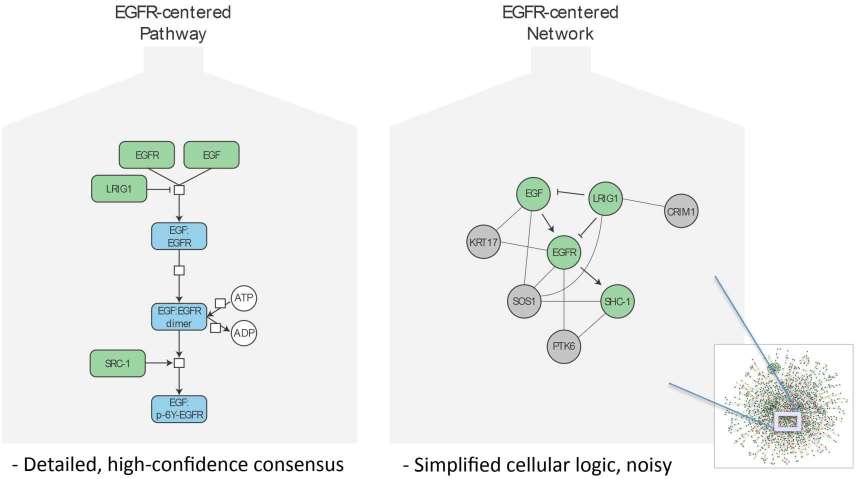
Biological Networks

- **Represent biological interactions**
  - ➡ Physical, regulatory, genetic, functional, etc.
- **Useful for discovering relationships in big data**
  - ➡ Better than tables in Excel
- **Visualize multiple heterogenous data types together**
  - ➡ See interesting patterns
- **Network analysis**
  - ➡ Well established quantitive metrics from graph theory

[Yeast, cell-cycle PPIs]

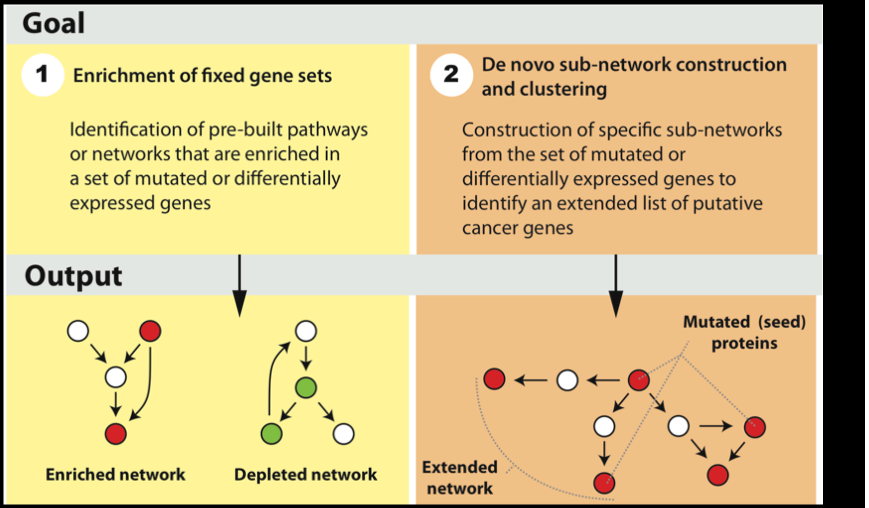


Pathways vs Networks



- Detailed, high-confidence consensus
- Biochemical reactions
- Small-scale, fewer genes
- Concentrated from decades of literature

- Simplified cellular logic, noisy
- Abstractions: directed, undirected
- Large-scale, genome-wide
- Constructed from *omics* data integration



Goal

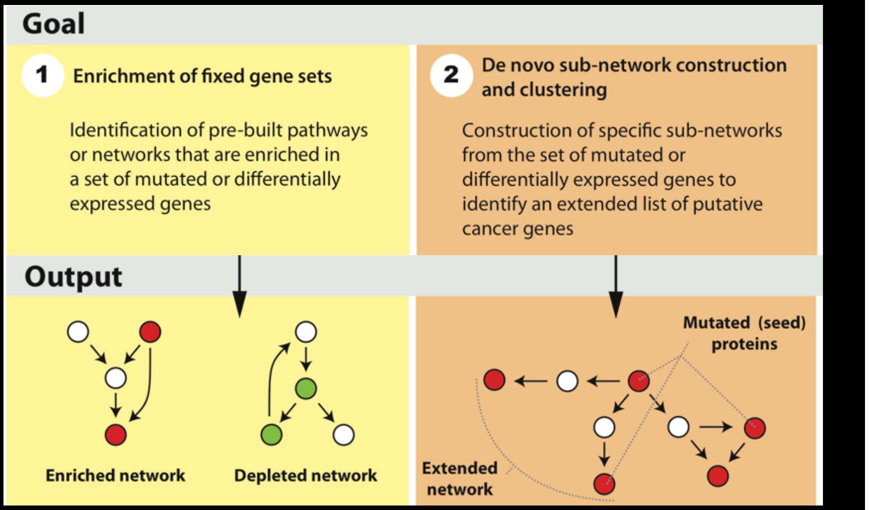
1 Enrichment of fixed gene sets

Identification of pre-built pathways or networks that are enriched in a set of mutated or differentially expressed genes

2 De novo sub-network construction and clustering

Construction of specific sub-networks from the set of mutated or differentially expressed genes to identify an extended list of putative cancer genes

Output



Goal

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Output

Enriched network

Depleted network

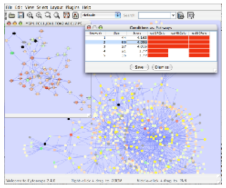
Mutated (seed) proteins

Extended network

What biological process is altered in this cancer?

Are NEW pathways altered in this cancer? Are there clinically relevant tumor subtypes?

Applications of Network Biology

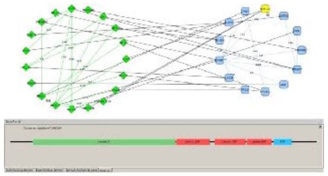


jActiveModules, UCSD

PathBlast, UCSD

- **Gene Function Prediction** – shows connections to sets of genes/proteins involved in same biological process
- **Detection of protein complexes/other modular structures** – discover modularity & higher order organization (motifs, feedback loops)
- **Network evolution** – biological process(es) conservation across species
- **Prediction of new interactions and functional associations** – Statistically significant domain-domain correlations in protein interaction network to predict protein-protein or genetic interaction; allostery in molecular networks

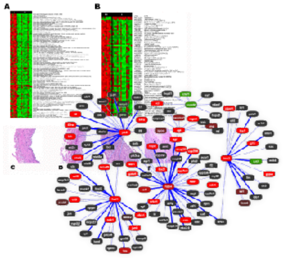
MCODE, University of Toronto



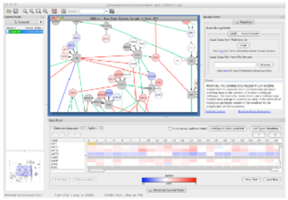
DomainGraph, Max Planck Institute

Slide from: humangenetics-amc.nl

Applications of Network Informatics in Disease

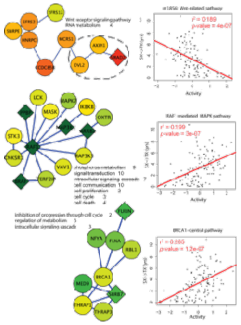


Agilent Literature Search



Mondrian, MSKCC

- **Identification of disease subnetworks** – identification of disease network subnetworks that are transcriptionally active in disease (disease sub-types).
- **Subnetwork-based diagnosis** – source of biomarkers for disease classification, identify interconnected genes whose aggregate expression levels are predictive of disease state
- **Subnetwork-based gene association** – map common pathway mechanisms affected by collection of genotypes

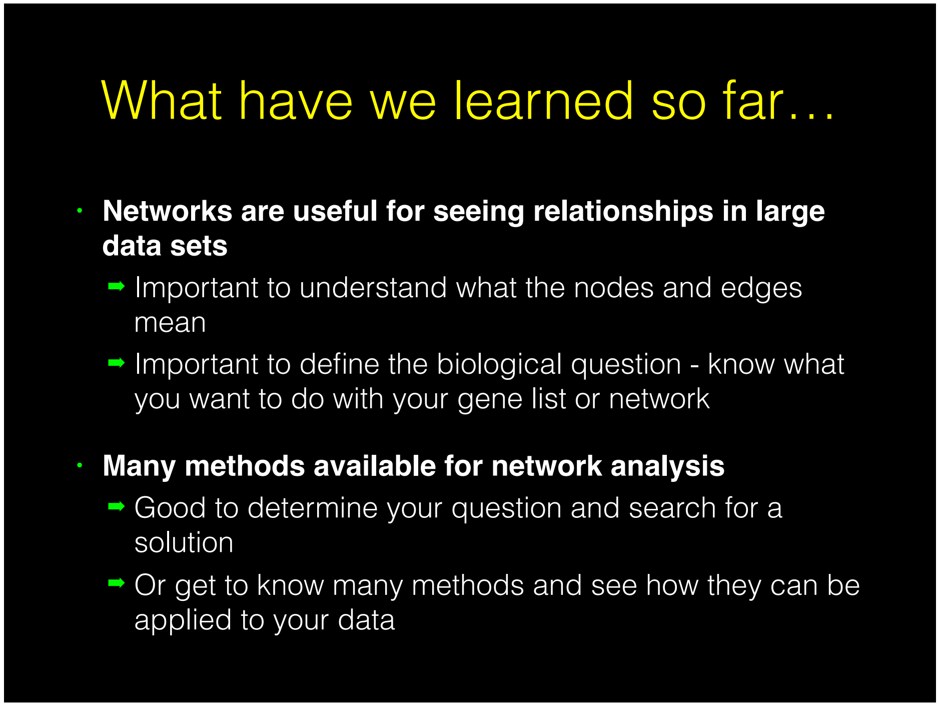


PinnacleZ, UCSD

Slide from: humangenetics-amc.nl

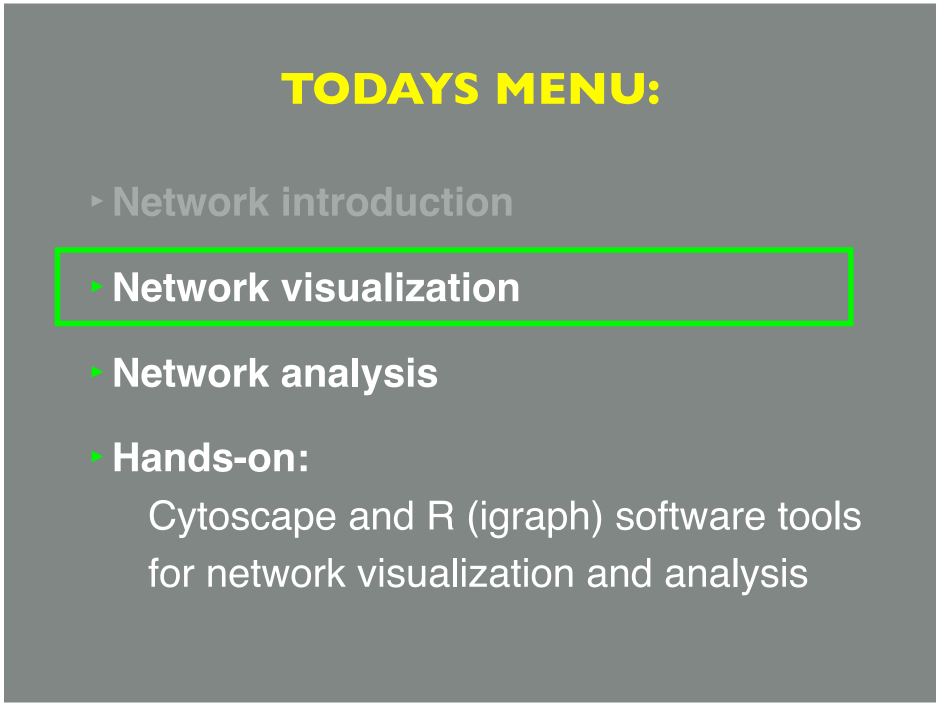
What's missing

- **Dynamics**
  - ➡ Pathways/networks represented as static processes
  - ➡ Difficult to represent a calcium wave or a feedback loop
  - ➡ More detailed mathematical representations exist that handle these e.g. Stoichiometric modeling, Kinetic modeling (VirtualCell, E-cell, …)
- **Detail** – atomic structures & exclusivity of interactions.
- **Context** – cell type, developmental stage



What have we learned so far…

- **Networks are useful for seeing relationships in large data sets**
  - ➡ Important to understand what the nodes and edges mean
  - ➡ Important to define the biological question - know what you want to do with your gene list or network
- **Many methods available for network analysis**
  - ➡ Good to determine your question and search for a solution
  - ➡ Or get to know many methods and see how they can be applied to your data



TODAYS MENU:

- Network introduction
- **Network visualization**
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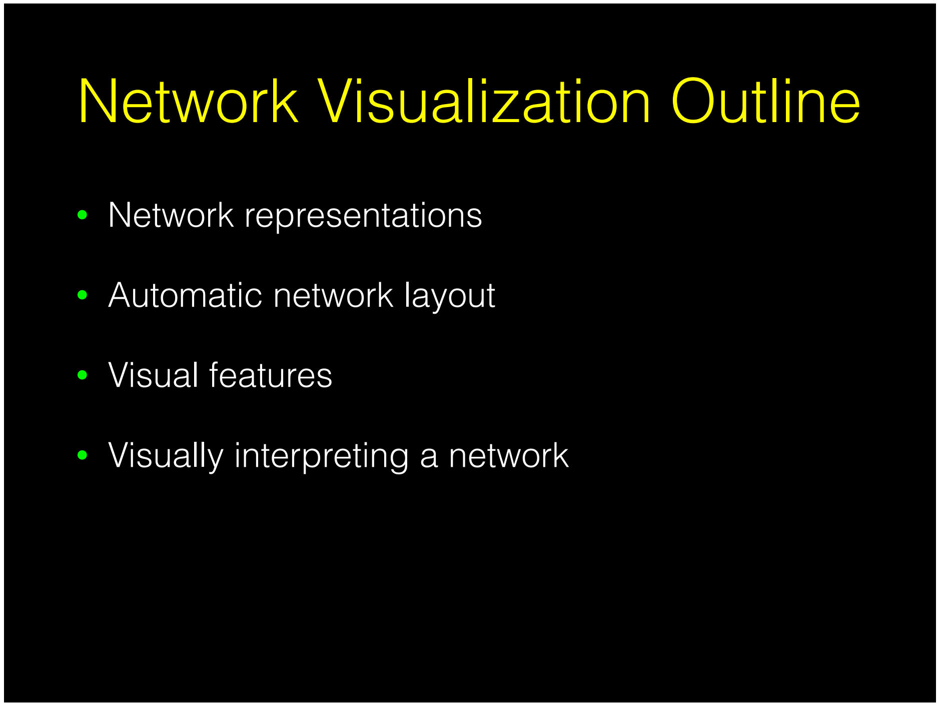
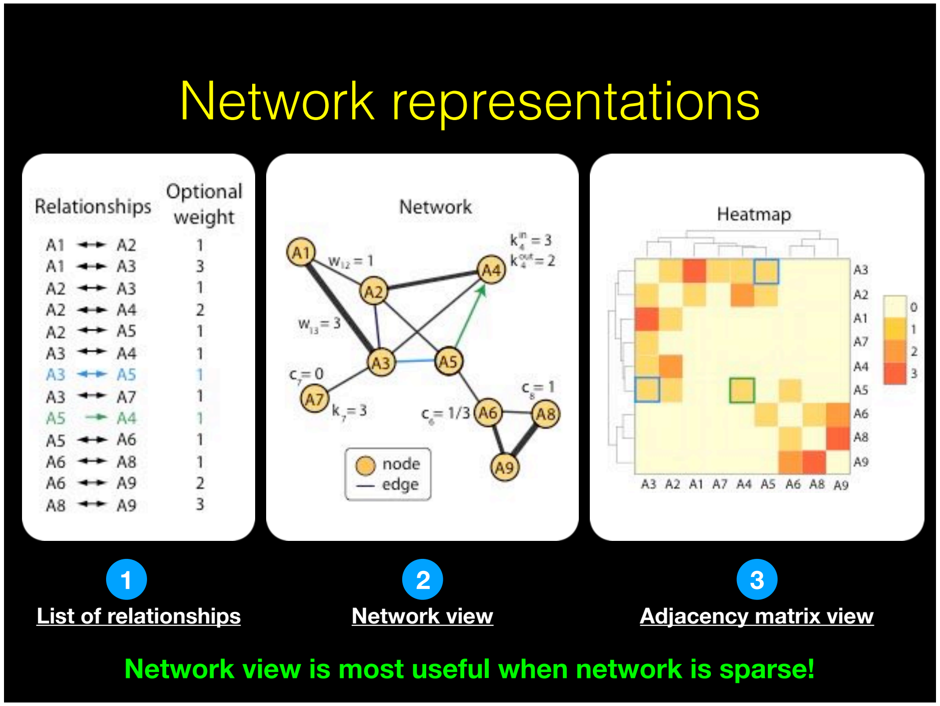
- **Hands-on:**

  Cytoscape and R (igraph) software tools for network visualization and analysis



Network Visualization Outline

- Network representations
- Automatic network layout
- Visual features
- Visually interpreting a network



Network representations

Relationships	Optional weight
A1 ↔ A2	1
A1 ↔ A3	3
A2 ↔ A3	1
A2 ↔ A4	2
A2 ↔ A5	1
A3 ↔ A4	1
A3 ↔ A5	1
A3 ↔ A7	1
A5 → A4	1
A5 ↔ A6	1
A6 ↔ A8	1
A6 ↔ A9	2
A8 ↔ A9	3

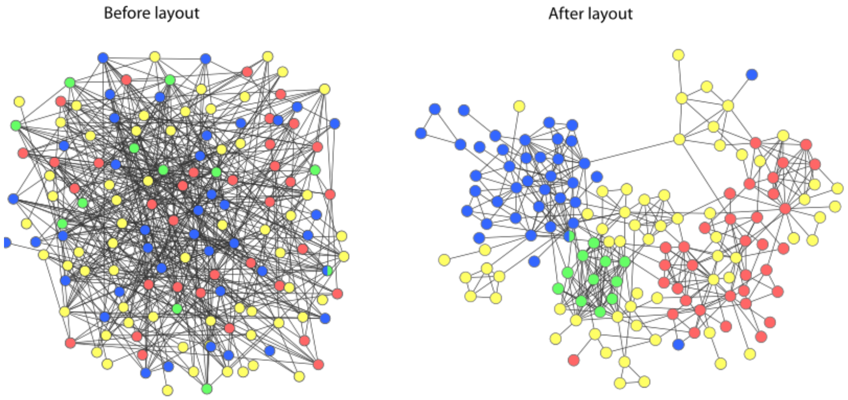
1 **List of relationships**

2 **Network view**

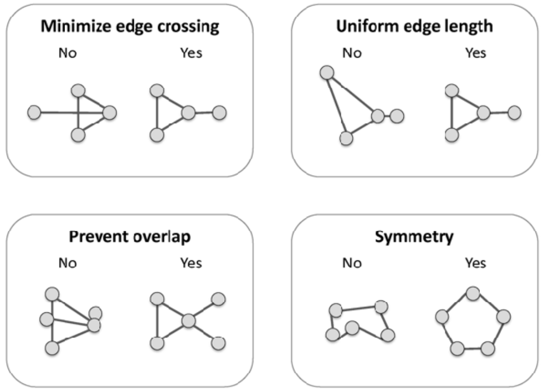
3 **Adjacency matrix view**

Network view is most useful when network is sparse!

Automatic network layout



- Modern **graph layouts** are optimized for speed and aesthetics. In particular, they seek to minimize overlaps and edge crossing, and ensure similar edge length across the graph.

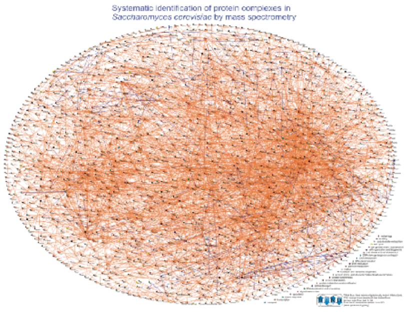


Force-directed layout:

Nodes repel and edges pull

- Good for up to 500 nodes
  - ➡ Bigger networks give hairballs
  - ➡ Reduce number of edges
  - ➡ Or just use a heatmap for dense networks
- Advice: try force directed first, or hierarchical for tree-like networks
- Tips for better looking networks
  - ➡ Manually adjust layout
  - ➡ Load network into a drawing program (e.g. Illustrator) and adjust labels

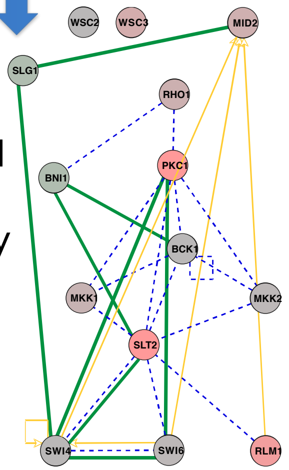
Dealing with 'hairballs': zoom or filter


Systematic identification of protein complexes in Saccharomyces cerevisiae by mass spectrometry

Zoom

Focus

PKC Cell Wall Integrity



━ **Synthetic Lethal**

━ **Transcription Factor Regulation**

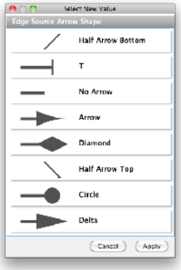
╍ **Protein-Protein Interaction**

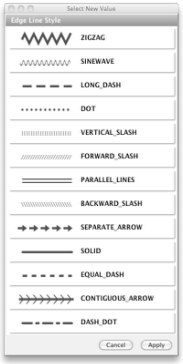
🔴 **Up Regulated Gene Expression**

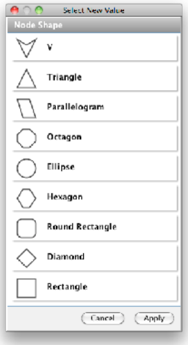
🟢 **Down Regulated Gene Expression**

Visual Features

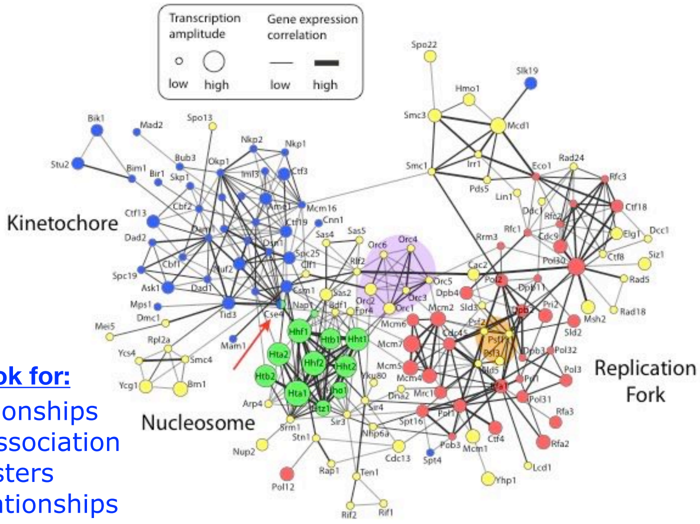
- Node and edge attributes
  - Text (string), integer, float, Boolean, list
  - E.g. represent gene, interaction attributes
- Visual attributes
  - Node, edge visual properties
  - Color, shape, size, borders, opacity...







Visually Interpreting a Network



What to look for:

Data relationships
Guilt-by-association
Dense clusters
Global relationships

What have we learned so far…

- Automatic layout is required to visualize networks
- Networks help you visualize interesting relationships in your data
- Avoid hairballs by focusing analysis
- Visual attributes enable multiple types of data to be shown at once – useful to see their relationships

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- Network introduction
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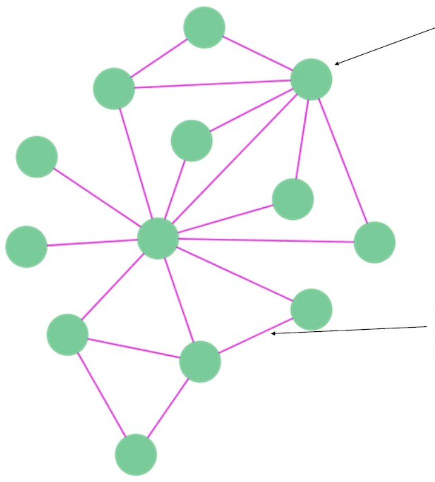
Introduction to graph theory

- Biological network analysis historically originated from the tools and concepts of social network analysis and the application of graph theory to the social sciences.
- Wikipedia defines graph theory as:
  - ➡ "[…] the study of graphs used to model pairwise relations between objects. A graph in this context is made up of **vertices** connected by **edges**".
- In practical terms, it is the set of concepts and methods that can be used to visualize and analyze networks

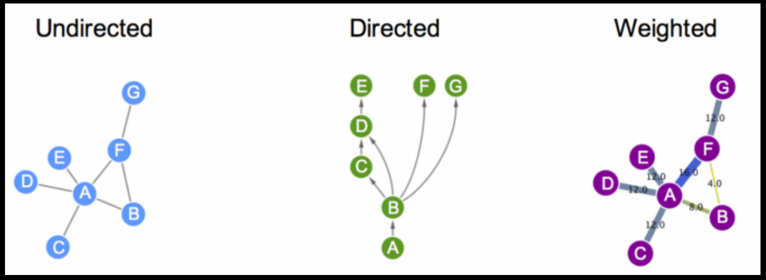
Network or graph



Node or vertex: protein, gene, drug, disease

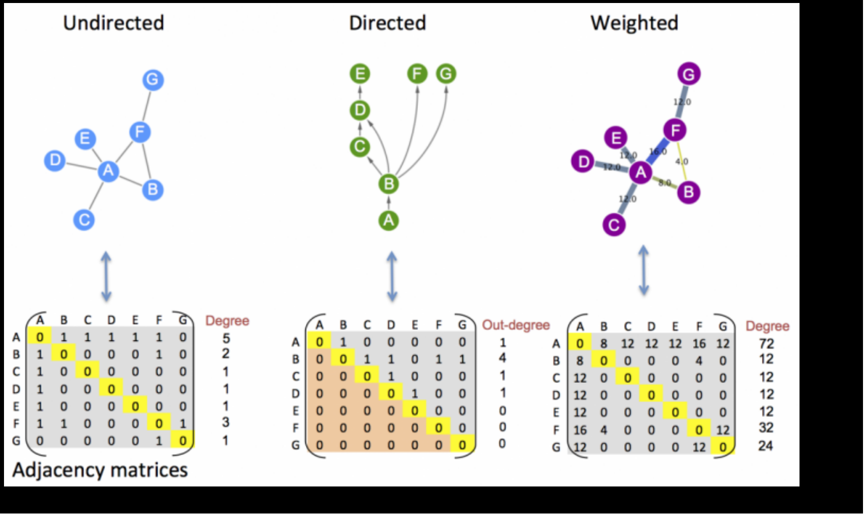
Edge or link: relation between nodes
- •Binary or continuous
- •Directed or indirected
- •Edge types

Types of network edges



Undirected	Directed	Weighted
Connection, without a given 'flow' implied (e.g. protein A binds protein B)	There is directional flow/signal implied (e.g. metabolic or gene networks)	Edges can also have weight (i.e. a 'strength' of interaction).

- Every network can be expressed mathematically in the form of an adjacency matrix



	A	B	C	D	E	F	G	Degree
A	0	1	1	1	1	1	0	5
B	1	0	0	0	0	1	0	2
C	1	0	0	0	0	0	0	1
D	1	0	0	0	0	0	0	1
E	1	0	0	0	0	0	0	1
F	1	1	0	0	0	0	1	3
G	0	0	0	0	0	1	0	1

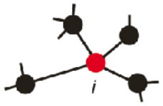
	A	B	C	D	E	F	G	Out-degree
A	0	1	0	0	0	0	0	1
B	0	0	1	1	0	1	1	4
C	0	0	0	1	0	0	0	1
D	0	0	0	0	1	0	0	1
E	0	0	0	0	0	0	0	0
F	0	0	0	0	0	0	0	0
G	0	0	0	0	0	0	0	0

	A	B	C	D	E	F	G	Degree
A	0	8	12	12	12	16	12	72
B	8	0	0	0	0	4	0	12
C	12	0	0	0	0	0	0	12
D	12	0	0	0	0	0	0	12
E	12	0	0	0	0	0	0	12
F	16	4	0	0	0	0	12	32
G	12	0	0	0	0	12	0	24

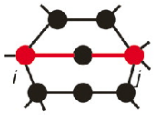
Adjacency matrices

Network topology

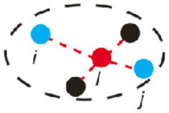
- Topology is the way in which the nodes and edges are arranged within a network.
- The most used topological properties and concepts include:
  - **Degree** (i.e. how may node neighbors)
  - **Communities** (i.e. clusters of well connected nodes)
  - **Shortest Paths** (i.e. shortest distance between 2 nodes)
  - **Centralities** (i.e. how 'central' is a given node?)
  - **Betweenness** (a measure of centrality based on shortest paths)



Degree k_i = number of links connected to node *i*



Distance d_{ij}= shortest path length between node *i* and *j*

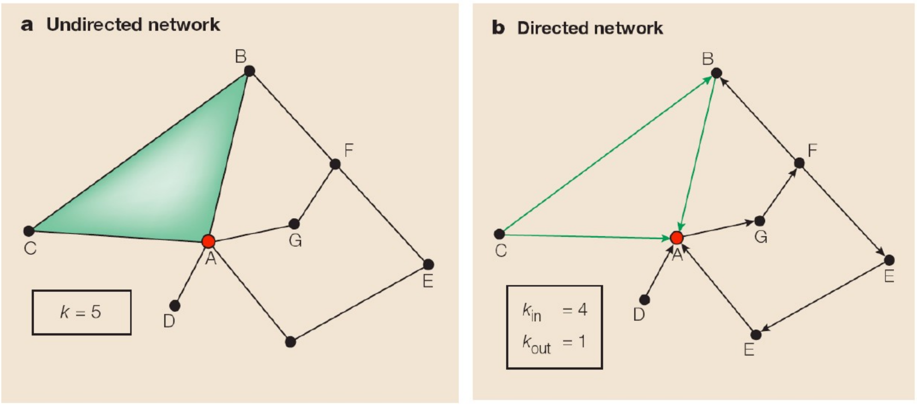


Betweenness $b_l = \sum_{ij} p_{ij}(l)/p_{ij}$

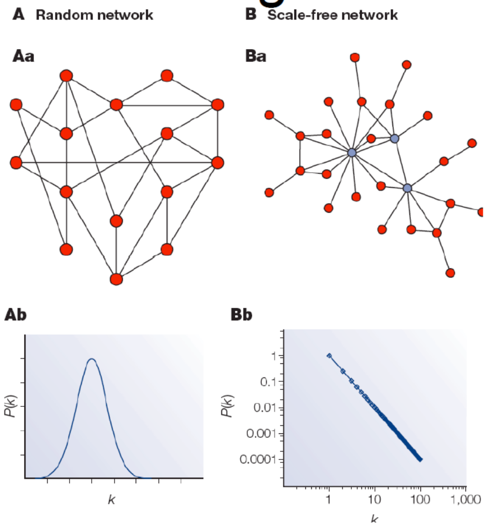
p_{ij} : number of shortest paths between *i* and *j*

$p_{ij}(l)$: number of shortest paths between *i* and *j* going through node *l*

Network Measures: Degree



Degree Distribution



P(k) is probability of each degree k, i.e fraction of nodes having that degree.

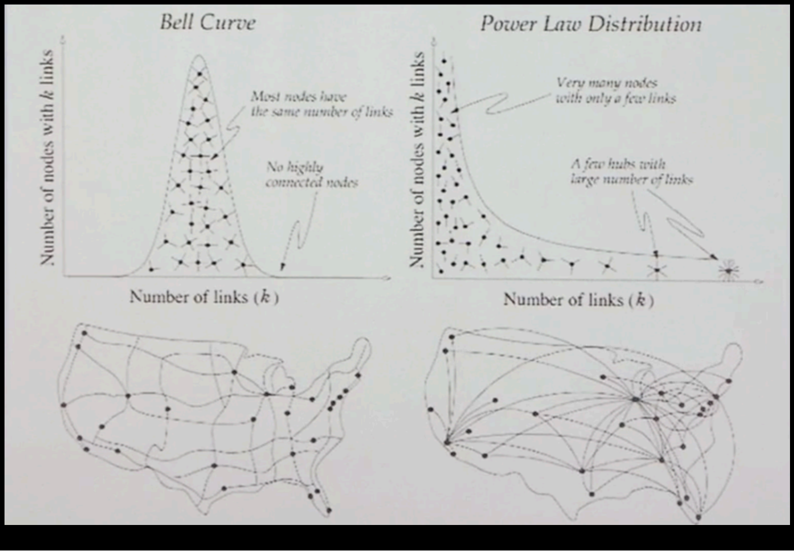
For random networks, P(k) is normally distributed.

For real networks the distribution is often a power-law:

$P(k) \sim k^{-\gamma}$

Such networks are said to be scale-free

Random graphs vs scale free



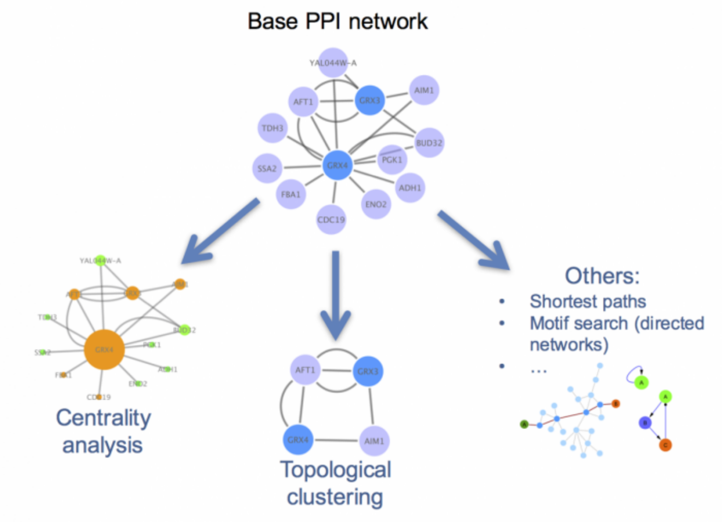
Scale-Free Networks are Robust

- Complex systems (cell, internet, social networks), are resilient to component failure
- Network topology plays an important role in this robustness
  - Even if ~80% of nodes fail, the remaining ~20% still maintain network connectivity
- *Attack vulnerability* if hubs are selectively targeted
- In yeast, only ~20% of proteins are lethal when deleted, and are 5 times more likely to have degree *k*>15 than *k*<5.

Implications

- Many biological networks (protein-protein interaction networks regulatory networks, etc…) are thought to have hubs, or nodes with high degree.
- For protein-protein interaction networks (PPIs) these hubs have been shown to be older [1] and more essential than random proteins [2]
  - [1] Fraser et al. *Science* (2002) 296:750
  - [2] Jeoung et al. *Nature* (2001) 411:41

Analyzing the topological features of a network is a useful way of identifying relevant participants and substructures that may be of biological significance.


Base PPI network

Centrality analysis

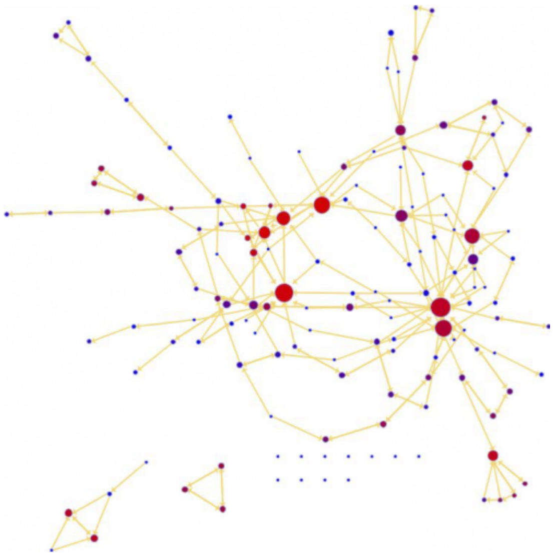
Topological clustering

Others:
- Shortest paths
- Motif search (directed networks)
- …

Centrality analysis

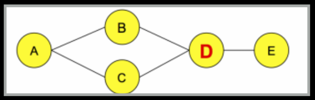
- Centrality gives an estimation on how important a node or edge is for the connectivity or the information flow of the network
- It is a useful parameter in signalling networks and it is often used when trying to find drug targets.
- Centrality analysis in PPINs usually aims to answer the following question:
  - ➡ Which protein is the most important and why?

Bigger, redder nodes have higher **centrality values** in this representation.



Betweenness centrality

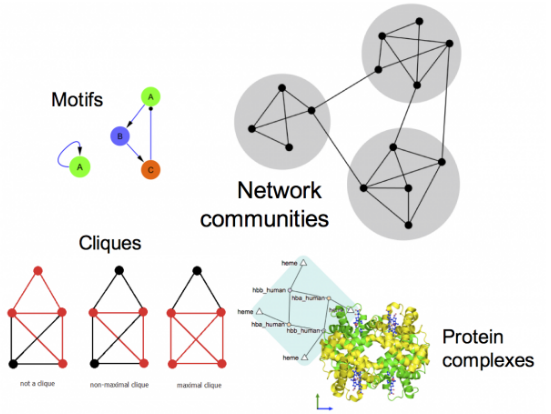
- Nodes with a high betweenness centrality are interesting because they lie on communication paths and can control information flow.
- The number of shortest paths in the graph that pass through the node divided by the total number of shortest paths.
- Betweenness centrality measures how often a node occurs on all shortest paths between two nodes.



Community analysis

- **Community**: A general, catch-all term that can be defined as a group (i.e. *cluster*) of nodes that are more connected within themselves than with the rest of the network. The precise definition for a community will depend on the method or algorithm used to define it.

Looking for communities in a network is a nice strategy for reducing network complexity and extracting functional modules (e.g. protein complexes) that reflect the biology of the network.



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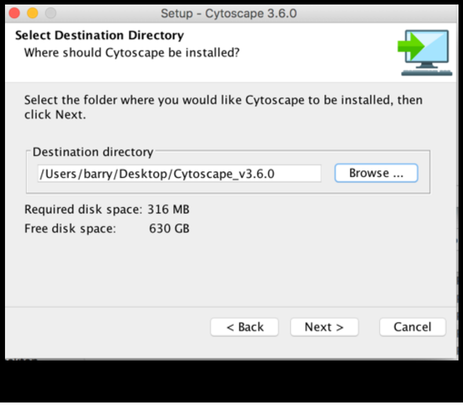
Practical issues

- Major tools for the **creation**, **manipulation** and **visualization** of biological networks include:
  - ➡ Cytoscape,
  - ➡ Gephi
  - ➡ R packages (igraph, graph, tidygraph)
- Tools for network analysis and modeling include:
  - ➡ Cytoscape apps/plugins
  - ➡ R packages (igraph and others)
  - ➡ NetworkX (for Python)
  - ➡ ByoDyn, COPASI

http://cytoscape.org/download.php

Note: If you are on a classroom Mac then be sure to install to your **Desktop** directory!


Setup - Cytoscape 3.6.0

Select Destination Directory

Where should Cytoscape be installed?

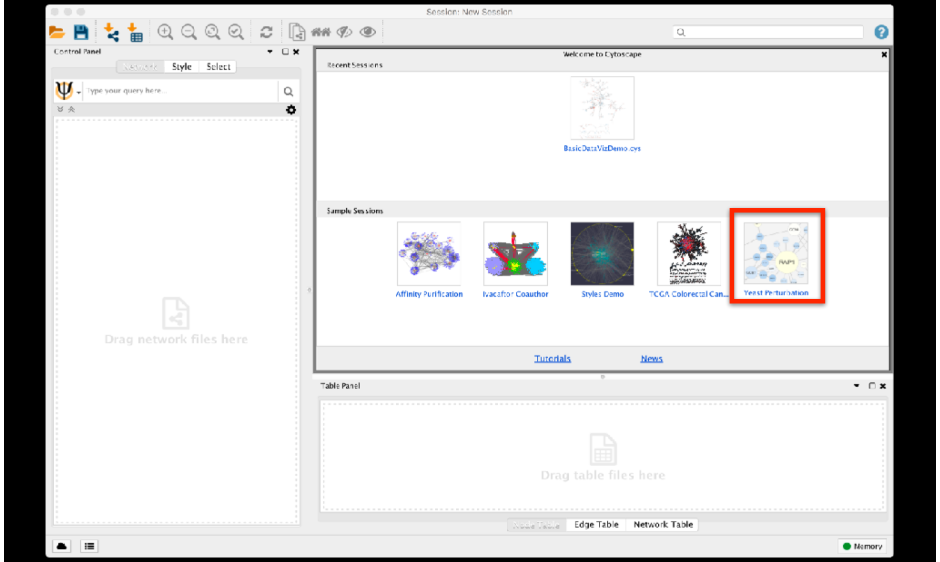
Select the folder where you would like Cytoscape to be installed, then click Next.

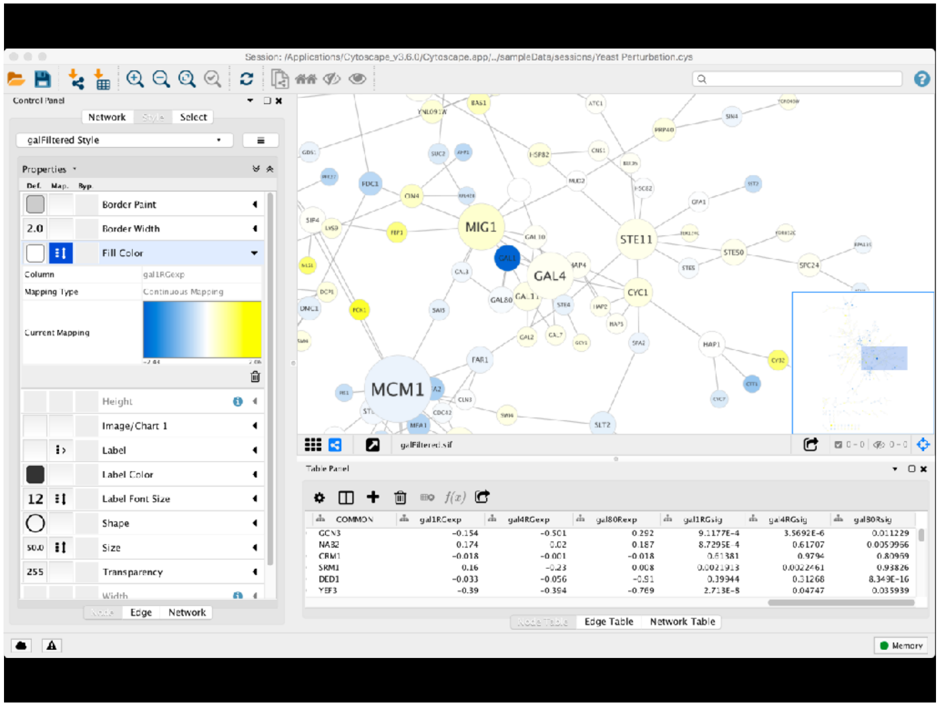
Destination directory

/Users/barry/Desktop/Cytoscape_v3.6.0

Required disk space: 316 MB

Free disk space: 630 GB





COMMON	gal1RGexp	gal4RGexp	gal80Rexp	gal1RGsig	gal4RGsig	gal80Rsig
GCN3	-0.154	-0.501	0.292	9.1177E-4	3.5692E-6	0.011229
NAB2	0.174	0.02	0.187	8.7295E-4	0.61707	0.0059956
CRM1	-0.018	-0.001	-0.018	0.61381	0.9794	0.80969
SRM1	0.16	-0.23	0.008	0.0021913	0.0022461	0.93826
DED1	-0.033	-0.056	-0.91	0.39944	0.31268	8.349E-16
YEF3	-0.39	-0.394	-0.769	2.713E-8	0.04747	0.035939

Cytoscape Memory Issues

- Cytoscape uses lots of memory and doesn't like to let go of it
  - ➡ An occasional restart when working with large networks is a good thing
  - ➡ Destroy views when you don't need them
- Since version 2.7, Cytoscape does a much better job at "guessing" good default memory sizes than previous versions but it still not great!
  - ➡ Java doesn't give us a good way to get the memory right at start time

Cytoscape Sessions

- Sessions save pretty much everything:
  - ➡ Networks
  - ➡ Properties
  - ➡ Visual styles
  - ➡ Screen sizes
- Saving a session on a large screen may require some resizing when opened on your laptop

Hands-on: Part 1

https://bioboot.github.io/bimm143_W18/lectures/#17

- The data used in **part 1** is from yeast, and the genes Gal1, Gal4, and Gal80 are all yeast transcription factors. The experiments all involve some perturbation of these transcription factor genes.
- In this network view, the following node attributes have been mapped to visual style properties in cytoscape:
  - ➡ The "gal80exp" expression values are used for Node Fill Color.
  - ➡ The Default Node Color, for nodes with no data mapping, is dark grey.
  - ➡ Nodes with expression values that are significant are rendered as rectangles, others are ovals.
  - ➡ The common name for each gene is used as the Node Label.

Hands-on: Part 2

https://bioboot.github.io/bimm143_W18/lectures/#17

- The data used in **part 2** is from an ocean metagenomic sequencing project - where all the genetic material in a sample of ocean water is sequenced.
- We will use the R package **igraph** and the bioconductor package **RCy3** together with Cytoscape.
- Many of these microbial species in these types of studies have not yet been characterized in the lab.
  - ➡ Thus, to know more about the organisms and their interactions, we can observe which ones occur at the same sites.
  - ➡ One way to do that is by using **co-occurrence networks** where you examine which organisms occur together at which sites.

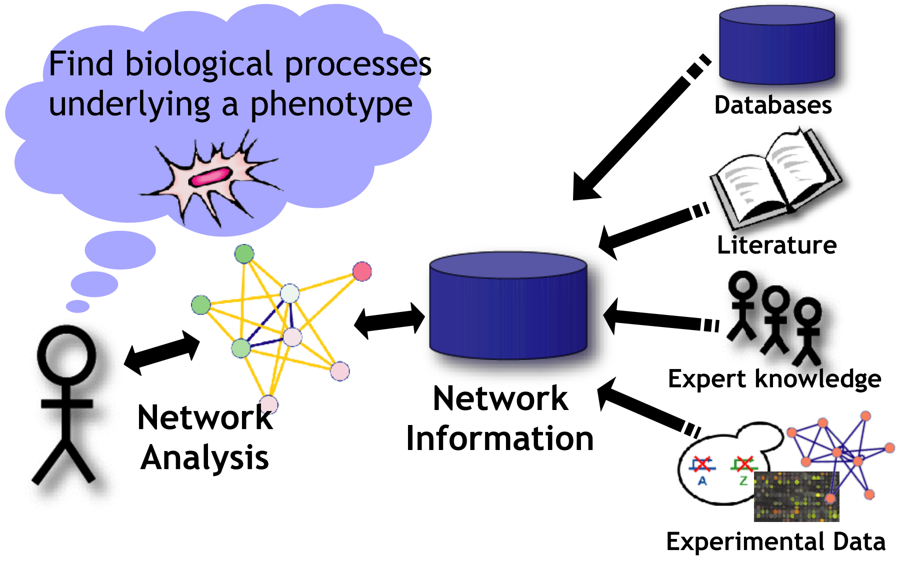
Summary

- Network biology makes use of the tools provided by **graph theory** to represent and analyze complex biological systems.
- Major types of biological networks include: genetic, metabolic, cell signaling etc.
- Networks are represented by **nodes** and **edges**.
- Biological networks have a number of characteristics, mainly:
  - ➡ **Scale-free**: A small number of nodes (hubs) are a lot more connected than the average node.
  - ➡ **Transitivity**: The networks contain communities of nodes that are more connected internally than they are to the rest of the network.
- Major tools for network analysis include: **Cytoscape**, **igraph**, Gephi and NetworkX.
- Two of the most used topological methods to analyze PPINs are:
  - ➡ **Centrality analysis**: Which identifies the most important nodes in a network, using different ways to calculate centrality.
  - ➡ **Community detection:** Which aims to find heavily inter-connected components that may represent protein complexes and machineries

Summary cont…

- **Cytoscape** is a useful, free software tool for network visualization and analysis
  - ➡ Provides basic network manipulation features
  - ➡ Plugins/Apps are available to extend the functionality
- The R **igraph** package has extensive network analysis functionality beyond that in Cytoscape
- The R bioconductor package **RCy3** package allows us to bring networks and associated data from R to Cytoscape so we can have the best of both worlds.

Network Analysis Overview



Find biological processes underlying a phenotype

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