

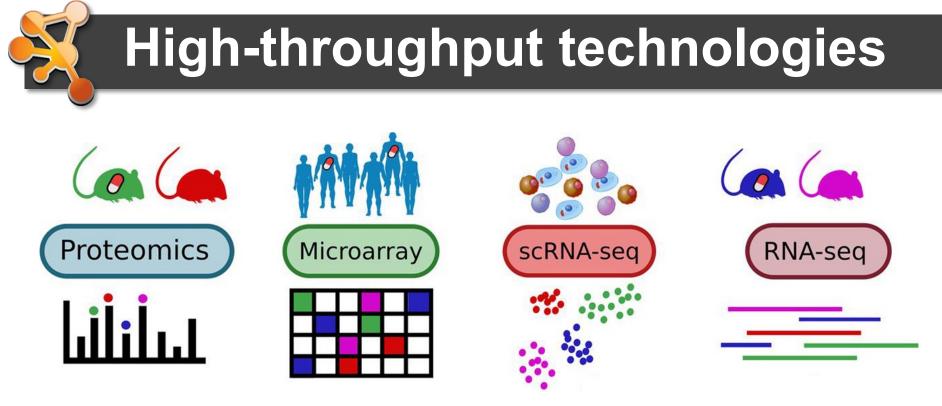


Introduction to Cytoscape and stringApp

Nadezhda T. Doncheva

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Statistical methods in bioinformatics, KU March 28th, 2023



- Enable us to characterize genome- and proteome-wide expression changes
- Usually result in hundreds of regulated molecular players (genes, proteins, etc.)
- It is challenging to derive relevant biological insights from 'omics data

Adapted from Griss et al., Mol & Cell Prot, 2020.



A typical data table

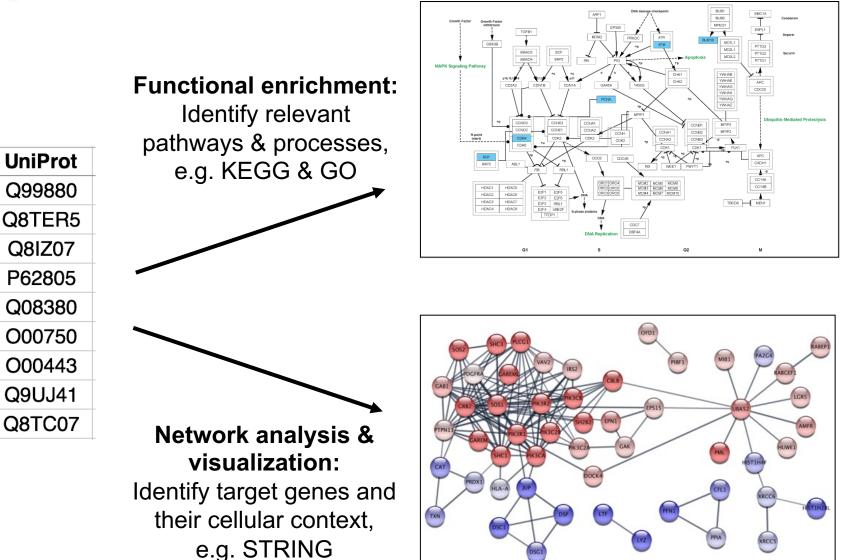
- Temporal analysis of the proteome of neuroblastoma cells in response to NGF (nerve growth factor) using mass spectrometry
- Identification of 78 proteins that interact with TrkA (tropomyosin-related kinase A) upon NGF stimulation

	А	В	С	D	G	J
1	UniProt	Gene name	Peptides	Sequence coverage [%]	5 min log ratio	10 min log ratio
2	Q99880	HIST1H2BL	5	35.7	-2.66	-2.66
3	Q8TER5	ARHGEF40	34	28.3	1.95	1.56
4	Q8IZ07	ANKRD13A	12	19.2	1.07	1.08
5	P62805	HIST1H4A	11	57.3	-2.31	-1.39
6	Q08380	LGALS3BP	14	28.2	-3.16	-2.98
7	O00750	PIK3C2B	35	24.2	2.21	2.31
8	O00443	PIK3C2A	29	17.8	1.13	1.26
9	Q9UJ41	RABGEF1	6	6.5	0.67	1.08
10	Q8TC07	TBC1D15	12	19.1	0.43	1.06

Emdal et al., Science Signaling, 2015

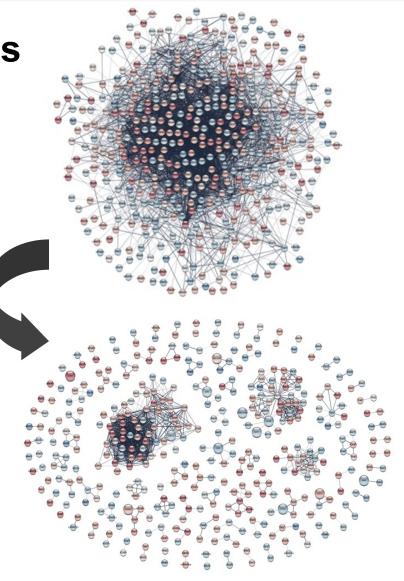


From gene lists to networks



Why use (biological) networks?

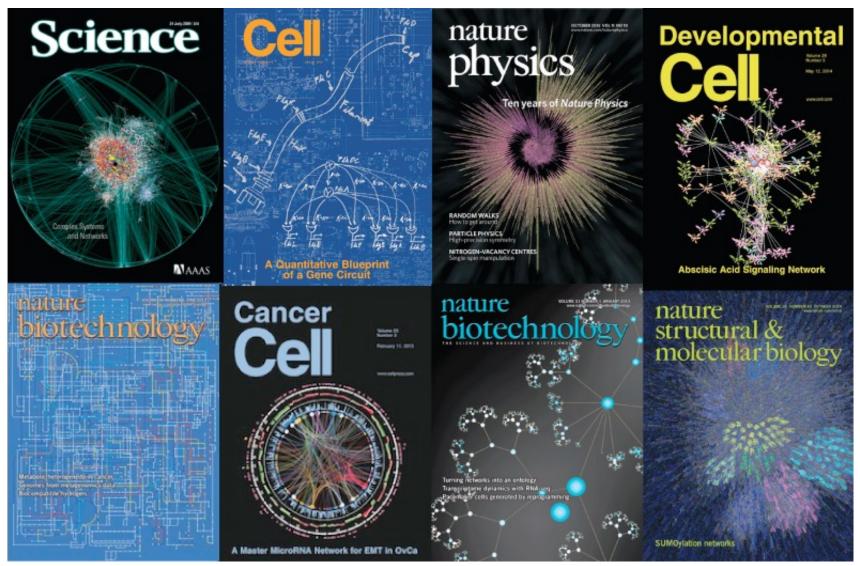
- Networks are powerful tools
 - ✓ More efficient than tables
 - ✓ Reduce complexity
 - ✓ Great for data integration
 - ✓ Intuitive visualization
- But also... Challenging!
- Network analysis and visualization techniques enable us to make the best use of networks.



Doncheva *et al.* (2019), *J Proteome Res*, 18(2): 623-632, Fig. 2 & Suppl. Fig. 1.



Applications in Research

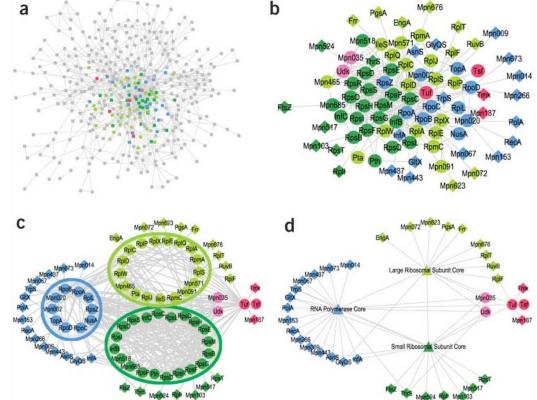




Omics data visualization

In 2010, a Nature Methods paper introduced major *themes* of network visualization:

- a) Mass spec analysis:
 400 PPI in pneumonia
 microbe
- b) Subnetwork with function annotations
- c) Layout with knowledge c of complexes
- d) Collapsed meta-nodes to reduce complexity

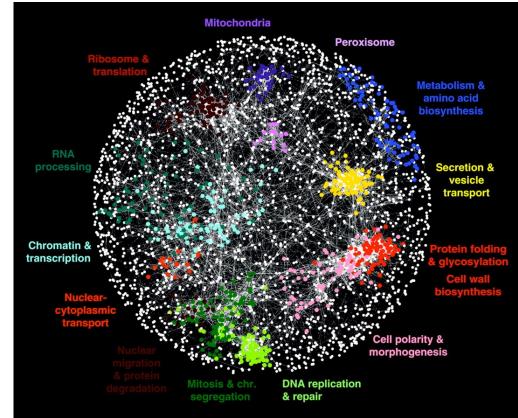


Gehlenborg *et al.* (2010) Visualization of omics data for systems biology. *Nature Methods*, 7, Fig. 1



Yeast genetic interactions

- Genetic interactions for the entire yeast genome
- Synthetic lethal screen of 1,712 genes resulting in 170,000 interactions
- Visualization of clustering within and between GO annotated functional groups
- Prediction of functions and identification of regulatory subnetworks

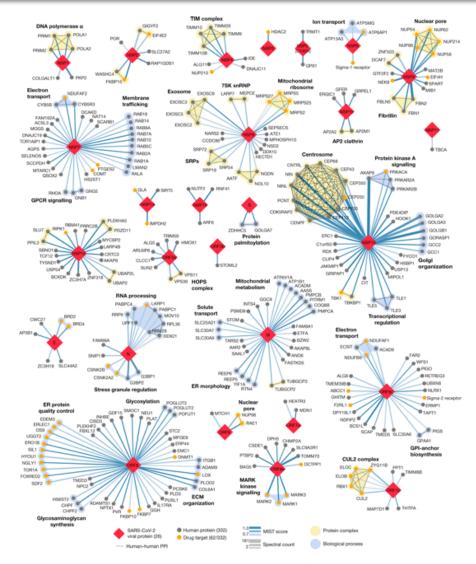


Costanzo *et al.* (2010) The genetic landscape of a cell. *Science*, 22, Fig. 1



SARS-CoV-2-human network

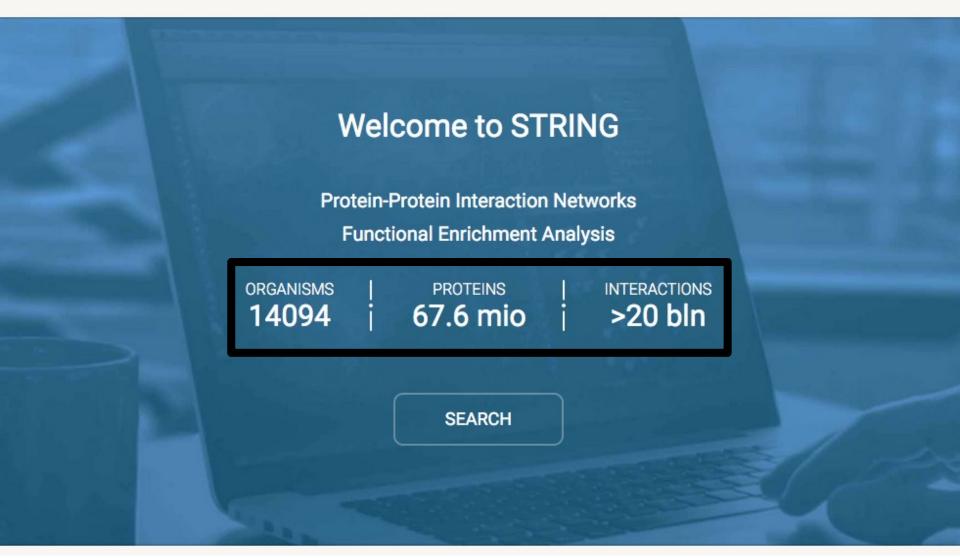
- AP-MS with 26 SARS-CoV-2 proteins reveals 332 interactions with human proteins
- Merged human-human physical protein interactions to identify complexes
- Used fill color to highlight known drug targets



Gordon *et al.* (2020): A SARS-CoV-2 protein interaction map reveals targets for drug repurposing. *Nature*, 583, Fig. 3

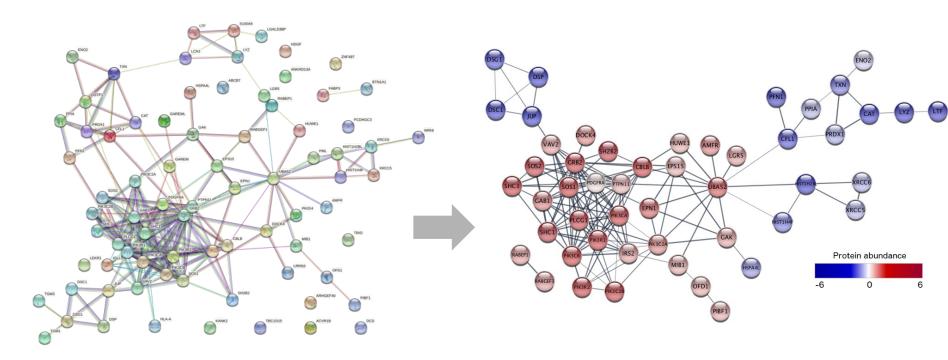
Do you already have some ideas, if and how you can use networks in your project(s)?







From STRING to Cytoscape

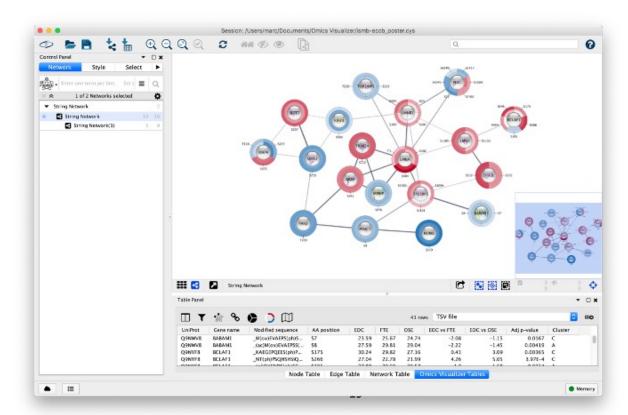


- Ideally, we want to be able to:
 - Integrate and easily show additional experimental data
 - Have more powerful analysis and visualization options
 - Create networks for large lists of genes



Cytoscape

- Open source tool for network analysis and visualization
- Large, active community of developers & users
- However, Cytoscape itself doesn't know any biology
- → Cytoscape apps: <u>apps.cytoscape.org</u>

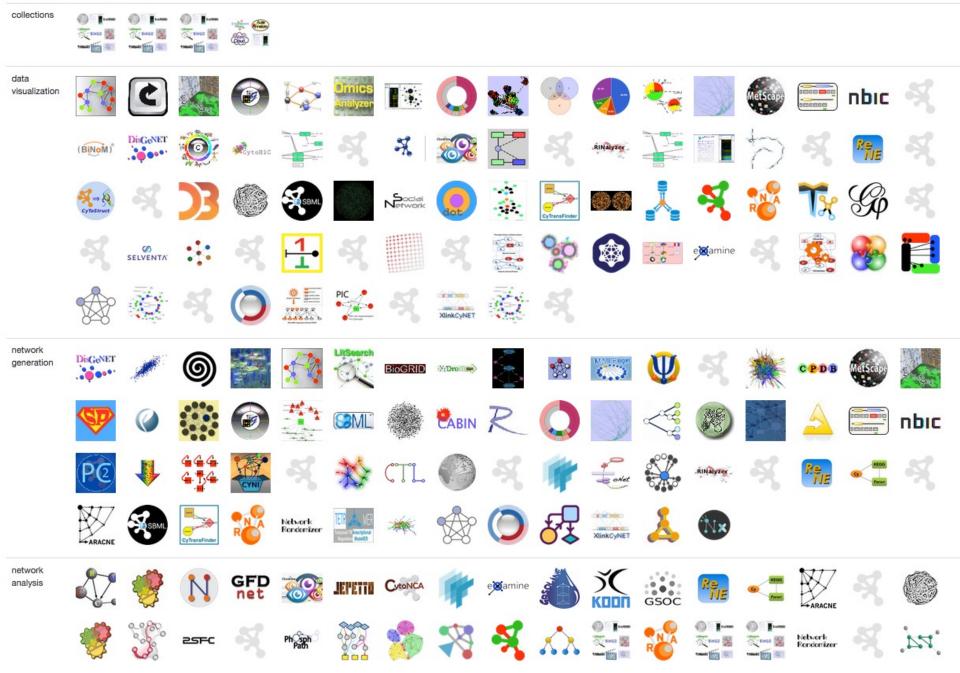


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Categories collections data visualization	BNMatch2	DKernel DKernel uses Diffusion Kernel
network generation network analysis graph analysis	optimized mapping between	algorithm to propagate sub-
online data import automation integrated analysis	BioGateway Cytoscape Plugin An explorative network building plugin that works with the	BETA: Build molecular interaction networks from IntAct database.
clustering systems biology utility enrichment analysis	CyCommunityDetection Integrates multiscale community detection and functional	dot-app Import/export of Graphviz files in Cytoscape
visualization data integration layout core app annotation	Top Downloaded App	more newest releases »
ontology analysis pathway database network comparison	ClueGO Creates and visualizes a functionally grouped network of	BINGO BINGO Calculates overrepresented GO terms in the network and display
more »	CluePedia: A ClueGO plugin for pathway insights using integrated	GeneMANIA Imports interaction networks from public databases from a list of
	stringApp import and augment Cytoscape networks from STRING	MCODE Clusters a given network based on topology to find densely

more top downloads »

Wall of Apps 372 total

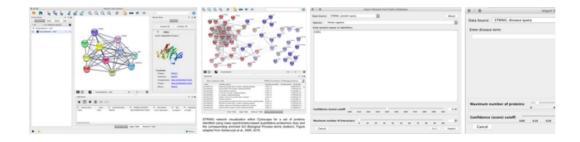




★★★★★ (28) 188212 downloads | citations | discussions 3.04

Details Release History

Categories: annotation, automation, data visualization, disease, enrichment analysis, gene-disease association, gene function prediction, import, interaction database, network generation, online data import, PPI-network, visualization



stringApp imports functional associations or physical interactions between protein-protein and protein-chemical pairs from **STRING**, **Viruses.STRING**, **STITCH**, **DISEASES** and from PubMed text mining into Cytoscape. Users provide a list of one or more gene, protein, compound, disease, or PubMed queries, the species, the network type, and a confidence score and *stringApp* queries the database to return the matching network. Currently, five different queries are supported:

- STRING: protein query -- enter a list of protein names (e.g. gene symbols or UniProt identifiers/accession numbers) to obtain a STRING network for the proteins
- STRING: PubMed query -- enter a PubMed query and utilize text mining to get a STRING network for the top N proteins associated with the query
- STRING: disease query -- enter a disease name to retrieve a STRING network of the top N
 proteins associated with the specified disease
- STITCH: protein/compound query -- enter a list of protein or compound names to obtain a network for them from STITCH
- STRING: cross-species query -- choose two species to obtain a STRING network between and within the proteins of the interacting species

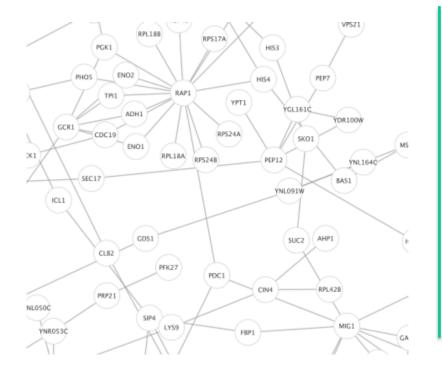




- Website
- Tutorial
- Cite this App
- Code Repository
- Automation Support
- 🖾 E-mail



Cytoscape core concepts



Node	Table	-



G		f(x)	-5	È+		
-	1.4			1.4	1100	

name 🔒	Degree	A COMMON	🚠 gal1RGexp	dal1RGsig
YDL194W	1	SNF3	0.139	0.018043
YDR277C	2	MTH1	0.243	2.186E-5
YBR043C	1	YBR043C	0.454	5.373E-8
YPR145W	1	ASN1	-0.195	3.174E-5
YER054C	2	GIP2	0.057	0.16958
YBR045C	3	GIP1	0.786	5.5911E-6
YBL079W	1	NUP170	-0.186	2.5668E-4
YLR345W	1	YLR345W	0.108	0.012373
YIL052C	1	RPL34B	-0.258	3.7855E-5

Networks e.g., protein-protein interaction networks

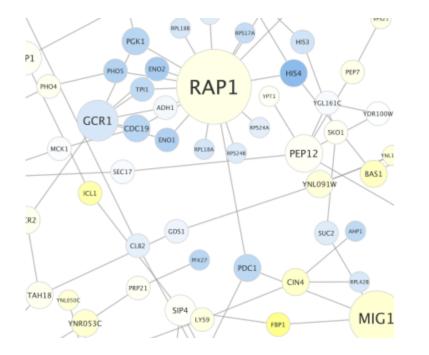
Tables

e.g., actual network data or annotations

Visual Styles



Cytoscape core concepts



Node	Table	•

۰	Ø	Þ	f(x)	-5	È,

🗎 name	Degree	A COMMON	📥 gal1RGexp	🚠 gal1RGsig
YDL194W	1	SNF3	0.139	0.018043
YDR277C	2	MTH1	0.243	2.186E-5
YBR043C	1	YBR043C	0.454	5.373E-8
YPR145W	1	ASN1	-0.195	3.174E-5
YER054C	2	GIP2	0.057	0.16958
YBR045C	3	GIP1	0.786	5.5911E-6
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Networks

e.g., protein-protein interaction networks

Tables

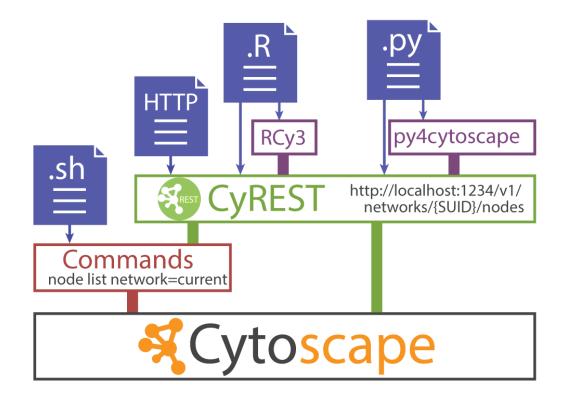
e.g., actual network data or annotations

Visual Styles



Cytoscape automation

 Use commands from R, Python, or JavaScript to execute Cytoscape, stringApp, and other apps' functionality



https://github.com/cytoscape/cytoscape-automation/wiki

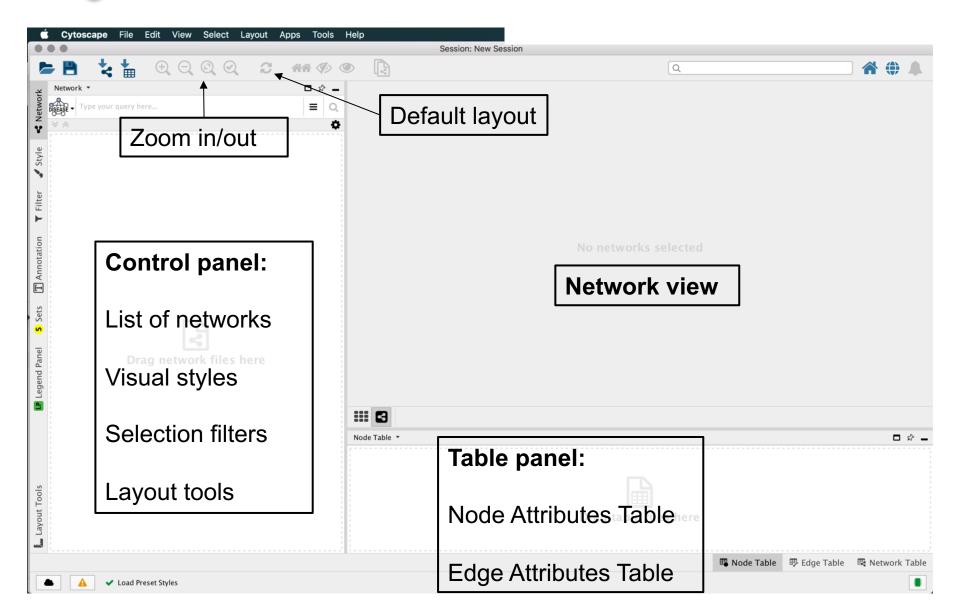


Let's try it out!

How many have installed Cytoscape 3.9.1?

If not installed yet, get it from here: <u>http://cytoscape.org/download.php</u>







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	nstall Apps Currently I	nstalled Check for Updates	
Download Site: Cy	rtoscape App Store	0	Manage Sites
QstringApp			8
all apps (248) collections (4) apps by tag	stringApp	stringApp 2.0.1	ugment Cytoscape m STRING
Install from File		View on App Store	Install
			Close

1



stringApp exercise 1

In this exercise, we will perform some simple queries to retrieve molecular networks in Cytoscape using the stringApp

1.1 Protein queries

Go to File \rightarrow Import \rightarrow Network from Public Databases. In the import dialog, choose STRING: protein query as Data Source and type your favorite protein into the Enter protein names or identifiers field (e.g. SORCS2).

Question 1: How many nodes and edges are in the resulting network? What types of information does the **Node Table** provide?

1.2 Disease queries

Go to File \rightarrow Import \rightarrow Network from Public Databases. In the import dialog, choose STRING: disease query as Data Source and type a disease of interest into the Enter disease term field (e.g. Alzheimer's disease). The next dialog selects the first of all the matches that the stringApp finds for your disease query. Select the intended disease and press Import to continue.

Question 2: Which additional attribute column do you get in the **Node Table** for a disease query compared to a protein query? Hint: check the last column.

Questions?

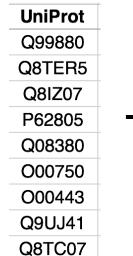
Lunch break!



From tables to styled networks

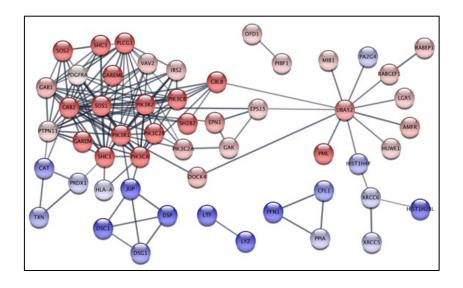
	А	В	С	D	D G	
1	UniProt	Gene name	Peptides	Sequence coverage [%]	5 min log ratio	10 min log ratio
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8	O00443	PIK3C2A	29	17.8	1.13	1.26
9	Q9UJ41	RABGEF1	6	6.5	0.67	1.08
10	Q8TC07	TBC1D15	12	19.1	0.43	1.06

Emdal et al., Science Signaling, 2015



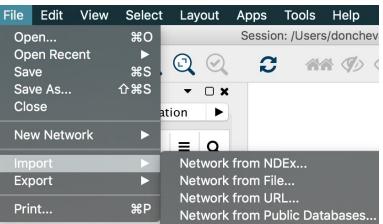


stringApp



Import networks in Cytoscape

- Starting with a list of genes and no network data
 - stringApp
 - IntAct app

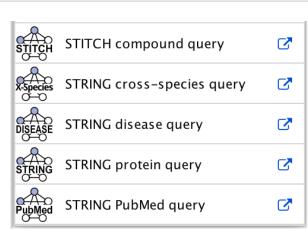


- Starting with a pathway of interest
 - KEGGscape app
 - ReactomeFI app
 - WikiPathways app
- Starting with your own network data
 - from files, e.g. Excel tables or text files
 - from R or Python via automation

stringApp

- STRING protein query
 - Queries for STRING interactions for one protein or for a list of identifiers
- STRING compound query
 - Queries for protein-compound interactions
- STRING disease query
 - Queries for disease-associated proteins from DISEASES and for STRING interactions between them
- STRING **PubMed** query
 - Retrieves STRING interactions for proteins co-occurring with the query term in PubMed
- STRING cross-species query
 - retrieves STRING interactions between and within the proteins of two interacting species

33



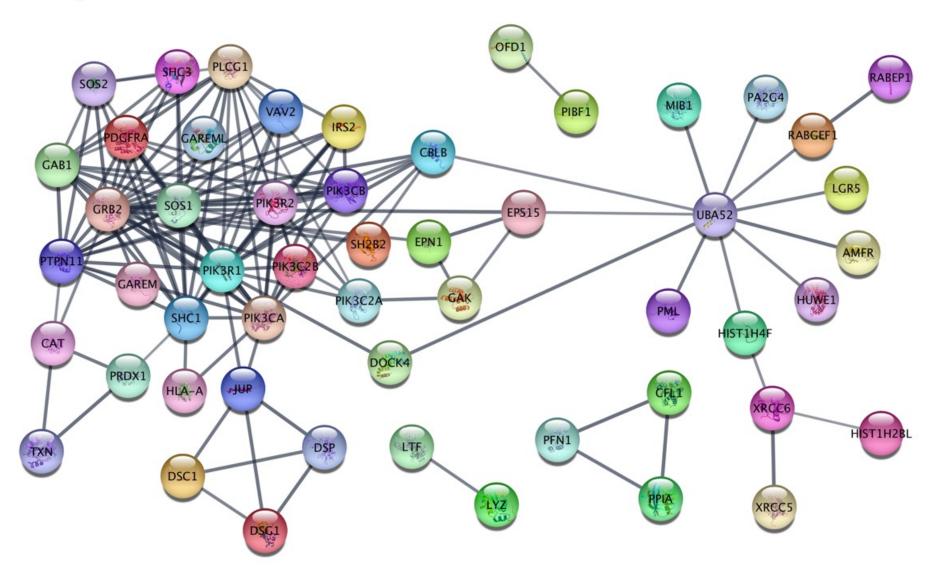








STRING network in Cytoscape





Node table (attributes)

- O X

Nodes (and edges) can have data associated with them, here protein information from STRING database

Table Panel

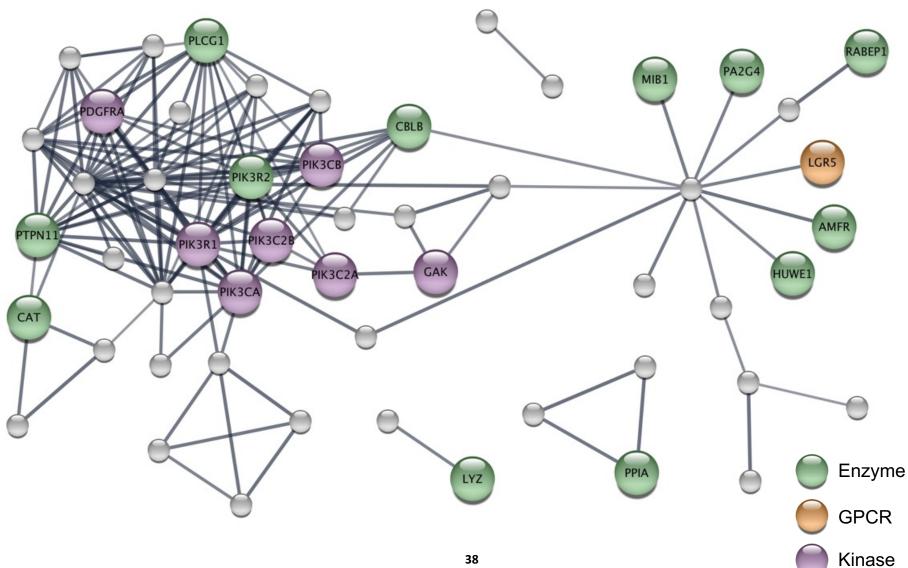
	S stringdb	S stringdb	S stringdb	S stringdb	С	compartment	C compartment		tissue
🚡 display name	🚠 canonical name	description	🚠 sequence	🚠 species		cytoskeleton	🚠 cytosol	-	blood
PHF1	O43189	Polycomb-like prot	MAQPPRLSRSGAS	Homo sapiens		5.0	0.326524		0.766667
EDAR	Q9UNE0	Tumor necrosis fac	MAHVGDCTQTPW	Homo sapiens			0.328125		0.750488
IL6	P05231	B-cell stimulatory f	MNSFSTSAFGPVA	Homo sapiens		2.617751	2.977923		4.0
CREB1	P16220	Cyclic AMP-respon	MTMESGAENQQS	Homo sapiens		1.709787	1.861972		3.449199
MS4A5	Q9H3V2	Membrane-spanni	MDSSTAHSPVFLV	Homo sapiens					
YWHAQ	P27348	Tyrosine 3-monoo	MEKTELIQKAKLA	Homo sapiens		2.200642	4.573817		4.794277
AKT1	P31749	V-akt murine thym	MSDVAIVKEGWLH	Homo sapiens		4.742235	5.0		3.61311
ADAM10	O14672	Disintegrin and me	MVLLRVLILLLSWA	Homo sapiens		0.905751	0.670166		4.566774
BIN1	075514	Box-dependent my	MAEMGSKGVTAG	Homo sapiens		4.193255	4.589923		4.468784
NCSTN	Q92542	Nicastrin; Essential	MATAGGGSGADP	Homo sapiens		2.584858	0.28125		1.411382
NRGN	Q92686	Neurogranin (prote	MDCCTENACSKP	Homo sapiens		1.019197	4.181165		2.951829
GIG25	Q6NSC9	Serpin peptidase in	MERMLPLLALGLL	Homo sapiens		2.315754	1.121397		3.634819
SYP	P08247	Major synaptic vesi	MLLLADMDVVNQ	Homo sapiens		3.11418	1.395096		1.911957

ode Table Edge Table Network Table

- Subcellular localization scores (<u>https://compartments.jensenlab.org/</u>)
- TISSUES expression scores (<u>https://tissues.jensenlab.org/</u>)
- Drug target information (<u>https://pharos.nih.gov/</u>)



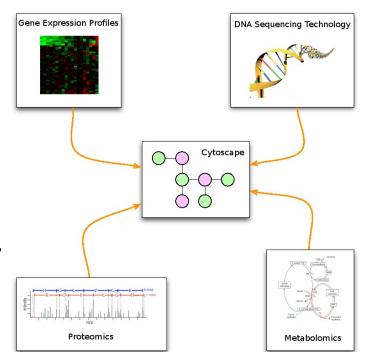
Pharos drug target information





Import attribute (table) data

- Import own data from files, e.g. Excel sheets, TSV, CSV
- Import data from public repositories, e.g. BioMart
- Import complex omics data via apps like Omics Visualizer
- Import data via automation from R or Python
- Identifiers have to match!





Know your identifiers

	А	В	С	D	G	J
1	UniProt	Gene name	Peptides	Sequence coverage [%]	5 min log ratio	10 min log ratio
2	Q99880	HIST1H2BL	5	35.7	-2.66	-2.66
3	Q8TER5	ARHGEF40	34	28.3	1.95	1.56
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9	Q9UJ41	RABGEF1	6	6.5	0.67	1.08
10	Q8TC07	TBC1D15	12	19.1	0.43	1.06

Table Panel

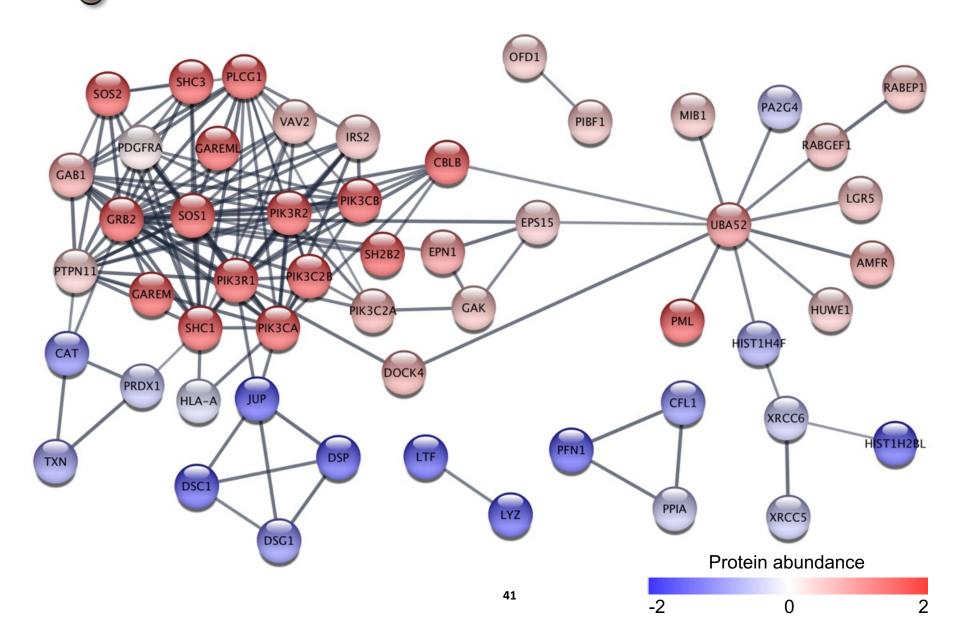
🌣 🛄 🕂 🛍 🎟 f(x)

📥 query term	name ^	description	📥 target family	📫 tissue nervous system	📥 5 min log ratio 📥	10 min log ratio
014976	GAK	cyclin G associated kinase	Kinase	5	0.38	0.94
P62993	GRB2	growth factor receptor-bound		5	2.39	2.52
Q99880	HIST1H2BL	histone cluster 1, H2bl		2	-2.66	-2.66
P62805	HIST1H4F	histone cluster 1, H4f		5	-2.31	-1.39
095757	HSPA4L	heat shock 70kDa protein 4-like		3	-1.93	-1.12
Q7Z6Z7	HUWE1	HECT, UBA and WWE domain co		5	0.1	0.82
Q9Y4H2	IRS2	insulin receptor substrate 2		4	0.28	0.97
P14923	JUP	junction plakoglobin		4	-2.59	-2.18
075473	LGR5	leucine-rich repeat containing	GPCR	3	0.61	1.0
P02788	LTF	lactotransferrin		4	-3.26	-2.39
P61626	LYZ	lysozyme		3	-3.96	-2.88
Q86YT6	MIB1	mindbomb E3 ubiquitin protei		5	-0.43	0.88
075665	OFD1	oral-facial-digital syndrome 1		4	-0.52	0.85
P16234	PDGFRA	platelet-derived growth factor	Kinase	5	0.71	0.3

Node Table Edge Table Network Table

- **#** ×

Expression data as node colors

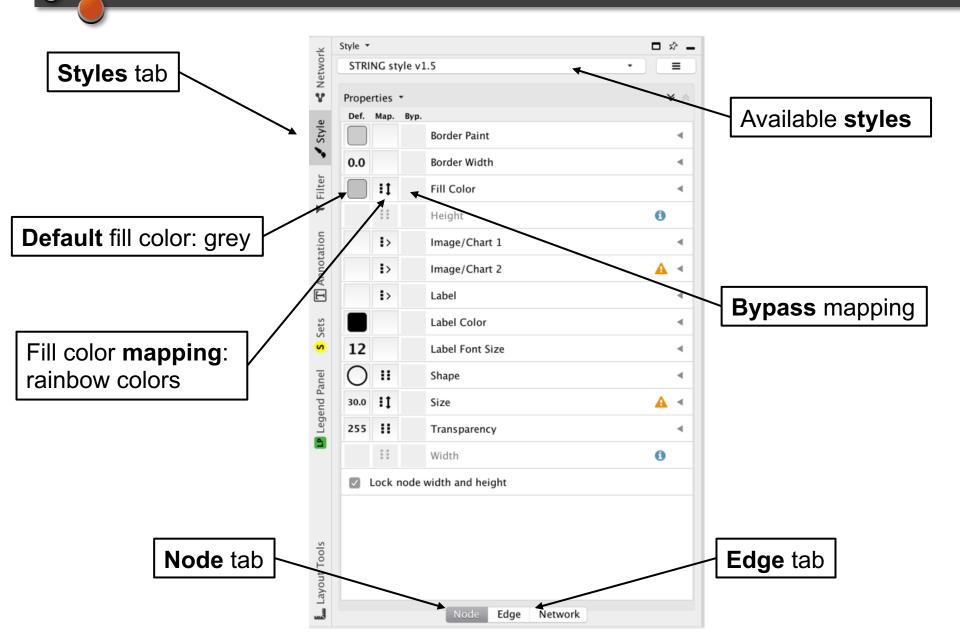




Visualize data using styles

- Visual attributes
 - Nodes: fill color, border color, border width, size, shape, opacity, label, etc.
 - Edges: line style, line color, line width, line opacity, ending type, ending color, etc.
- Mapping types
 - Continuous (numeric values)
 - Expression values, edge interaction scores
 - Discrete (categories)
 - Type of interaction, protein family
 - Pass-through (labels)
- Pre-defined visual styles

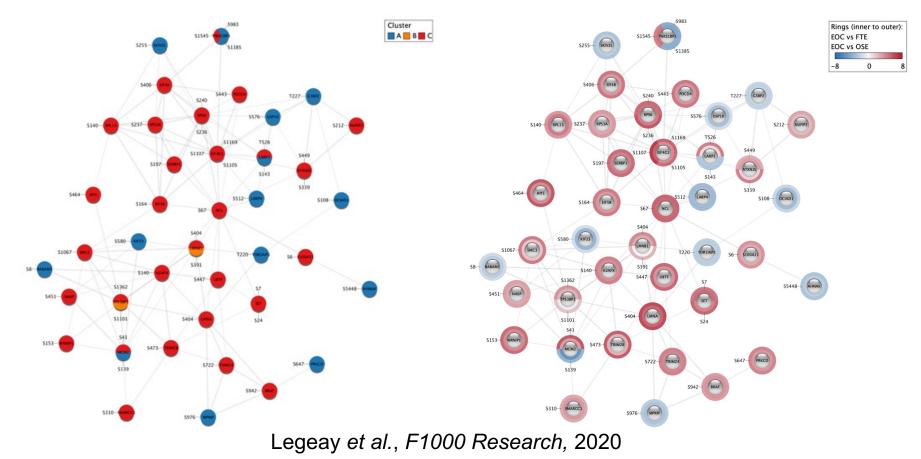
Styles: User interface





Omics Visualizer app

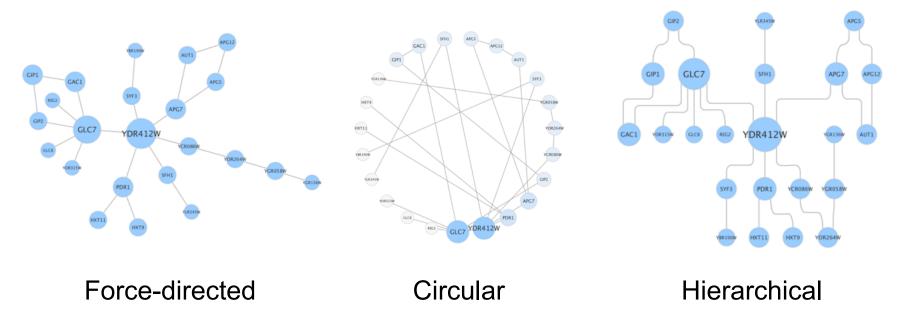
How to visualize multi-omics data, time series, or phosphoproteomics data, i.e. tables with multiple rows for each protein? \rightarrow OV creates pies inside or donuts around the nodes.





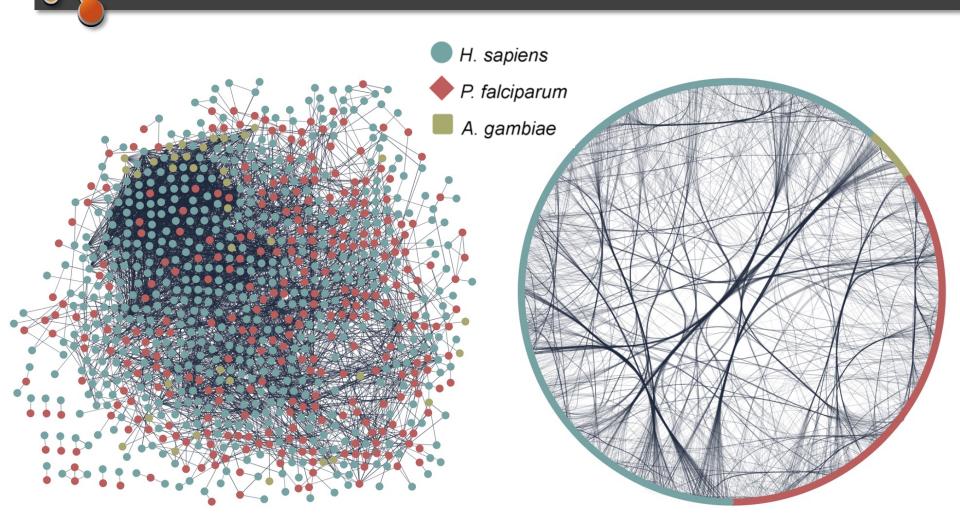
Layouts

- Layouts determine the location of nodes and (sometimes) the paths of edges
- Use them to convey the relationships between nodes
- Recommended apps: yFiles Layouts, layoutSaver



• There is not one *correct* layout \rightarrow Try different things!

Layout examples



Force-directed

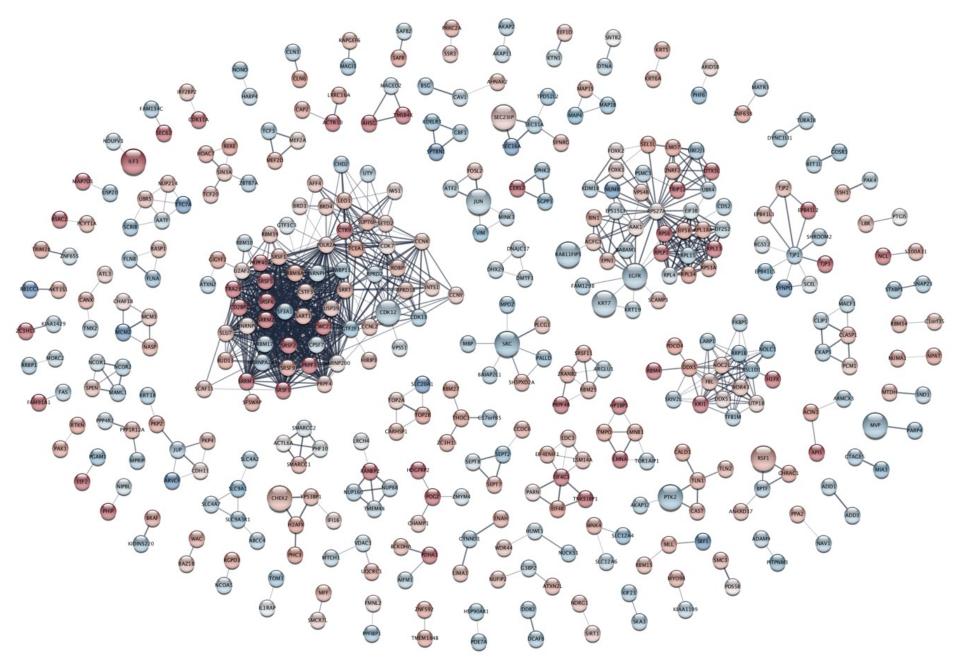
Circular

Created with stringApp 2.0



Network clustering

- Capture the structure of the network by identifying dense subgraphs, e.g.
 - Protein complexes in protein-protein interaction networks
 - Functional modules in functional association networks
 - Protein families in protein similarity networks
- Advantages for visualization → it can guide the layout of the network and be used for network simplification, e.g.
 - Represent each cluster as one node
 - Show only edges within clusters

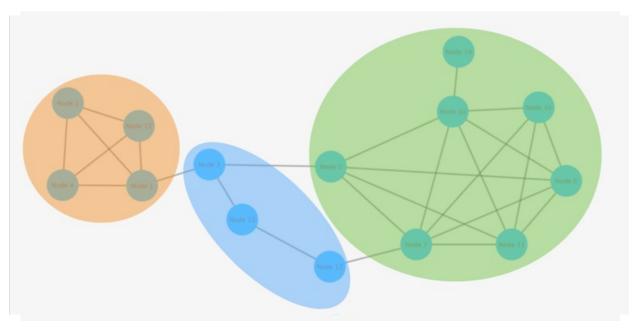


Doncheva et al., J Proteome Research, 2019



Network clustering example

- Group nodes together based on a measure of similarity between the nodes, e.g. edges or edge weights
- MCL (Markov CLustering)
 - Fast algorithm
 - No need to specify number of clusters





Clustering in Cytoscape

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clusterMaker2

Multi-algorithm clustering app for Cytoscape

★ ★ 🚖 🏠 (23) 89244 downloads | citations | discussions 🧕



Release History

Categories: automation, clustering, data visualization, gene expression, grouping, heat map visualization, visualization



clusterMaker2 is the Cytoscape 3 version of the clusterMaker plugin. clusterMaker2 provides several clustering algorithms for clustering data within columns as well as clustering nodes within a network. This version also provides support for two new algorithms: Fuzzy C-Means and a new "Fuzzifier". In addition to providing clustering algorithms, clusterMaker2 provides heatmap visualization of both node data and edge data as well as the ability to create new networks based on the results of a clustering algorithm.

Current node attribute algorithms:

- Hierarchical
- K-Means
- K-Medoid



Version 1.3.1 Released 30 Oct 2018 Works with Cytoscape 3.6 Download Stats Click here

🛓 Download

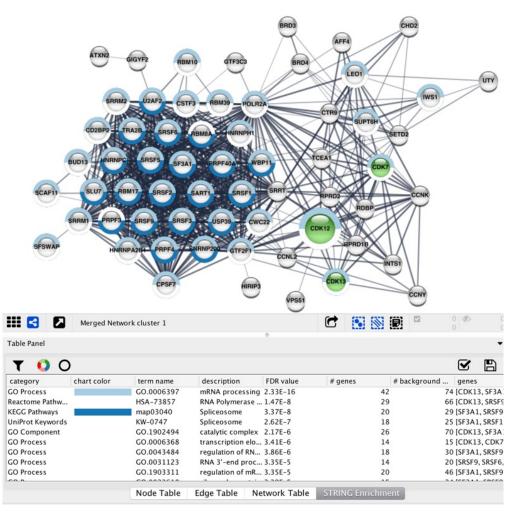


Ask a question

- Search BioStars
- Website
- Tutorial
- Cite this App
- Code Repository
- Automation Support
- 🖾 E-mail

stringApp functional enrichment

- Can be very useful for visualization
- Filter terms by category, remove redundant terms
- Visualize significant terms
- Many categories: Gene Ontology terms, Pathways, Diseases & phenotypes, Tissues & subcellular localization, Protein domains, Publications





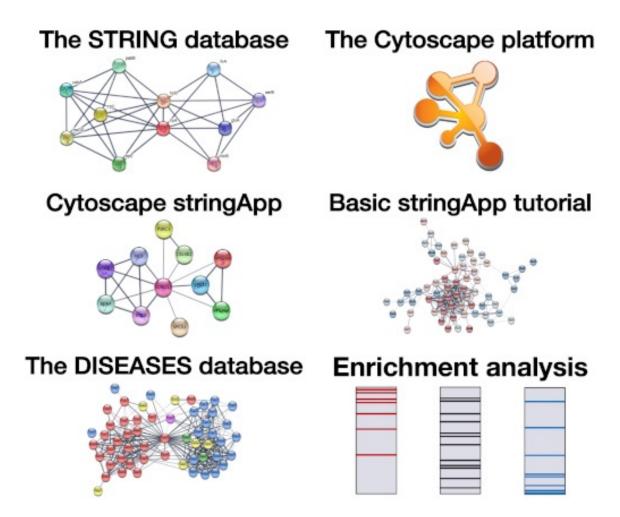
Save data

- Cytoscape sessions save everything (.cys files)
- Export networks in different formats
- Export node & edge tables as text files
- Publication quality graphics in several formats





Supporting lectures



https://www.youtube.com/c/LarsJuhlJensen



Tutorials & getting help

- STRING & stringApp:
 - YouTube videos: <u>https://www.youtube.com/c/LarsJuhlJensen</u>
 - Tutorials & exercises: <u>https://jensenlab.org/training/</u>
 - Automation repository: <u>https://github.com/scaramonche/EuBIC2020_Cytoscape</u>
- Cytoscape
 - Tutorials: <u>https://github.com/cytoscape/cytoscape-tutorials/wiki</u>
 - YouTube videos: <u>https://www.youtube.com/channel/UCv6auk9FK4NgXiXiqrDLccw</u>
 - Helpdesk mailing list: <u>cytoscape-helpdesk@googlegroups.com</u>
 - Publications using Cytoscape: <u>https://cytoscape-publications.tumblr.com/</u>
 - Automation: <u>https://github.com/cytoscape/cytoscape-automation/wiki</u>

Questions?



Hands-on exercises

- Continue with stringApp exercises
 - Exercises 2 to 5: <u>https://jensenlab.org/training/stringapp/</u>
- Work with your own data
 - Follow stringApp exercises but adapt them to your own data
- Try out one of the Cytoscape tutorials
 - Basic data visualization
 - RNA-seq data analysis
 - Differentially Expressed Genes
 - Using WikiPathways App
 - And many more...

https://github.com/cytoscape/cytoscape-tutorials/wiki



stringApp exercise 2

In this exercise, we will work with the list of proteins associated with epithelial ovarian cancer (EOC) in the study by <u>Francavilla *et al.*</u> to perform typical network import and visualization tasks.

2.1 Protein network retrieval & layout

Question 1: How many nodes and edges are there in the resulting network? Do the proteins all form a connected network? Why?

Question 2: Does any of the suggested layouts make patterns in the network easy to recognize? (Recommended: install the app **yFiles Layout Algorithms**)

2.2 Discrete color mapping

Question 3&4: How many of the proteins in the network are ion channels (IC) or GPCRs? How many kinases are in the network?

2.3 Data import

Question 5: Do you see the columns from the Excel table in the Node Table?

2.4 Continuous color mapping

Question 6: Are the up-regulated nodes grouped together?



stringApp exercise 3

In this exercise, we will work with the list of differentially abundant proteins from the study by <u>Francavilla *et al.*</u> and perform typical network analysis tasks.

Prerequisites: install the app ClusterMaker2

3.1 Network clustering

Question 1: How many clusters have at least 10 nodes?

3.2 Subnetworks

Question 2: How many nodes and edges are there in this cluster?

Question 3: How many edges does the resulting network contain and why are there now fewer edges?

3.3 Functional enrichment

Question 4: How many statistically significant terms are in the table? Which is the most significant term for each of the categories GO Biological Process, Reactome and KEGG Pathways?

3.4 Functional enrichment extras

Question 5: What is the title of the most recent publication?