Network Biology

BMI/CS 776

www.biostat.wisc.edu/bmi776/

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Goals for lecture

- Biological networks
- Challenges of integrating high-throughput assays
- Connecting relevant genes/proteins with interaction networks
- ResponseNet algorithm
- Evaluating pathway predictions
- Classes of signaling pathway prediction methods

High-throughput screening

- Which genes are involved in which cellular processes?
- Hit: gene that affects the phenotype
- Phenotypes include:
  - Growth rate
  - Cell death
  - Cell size
  - Intensity of some reporter
  - Many others

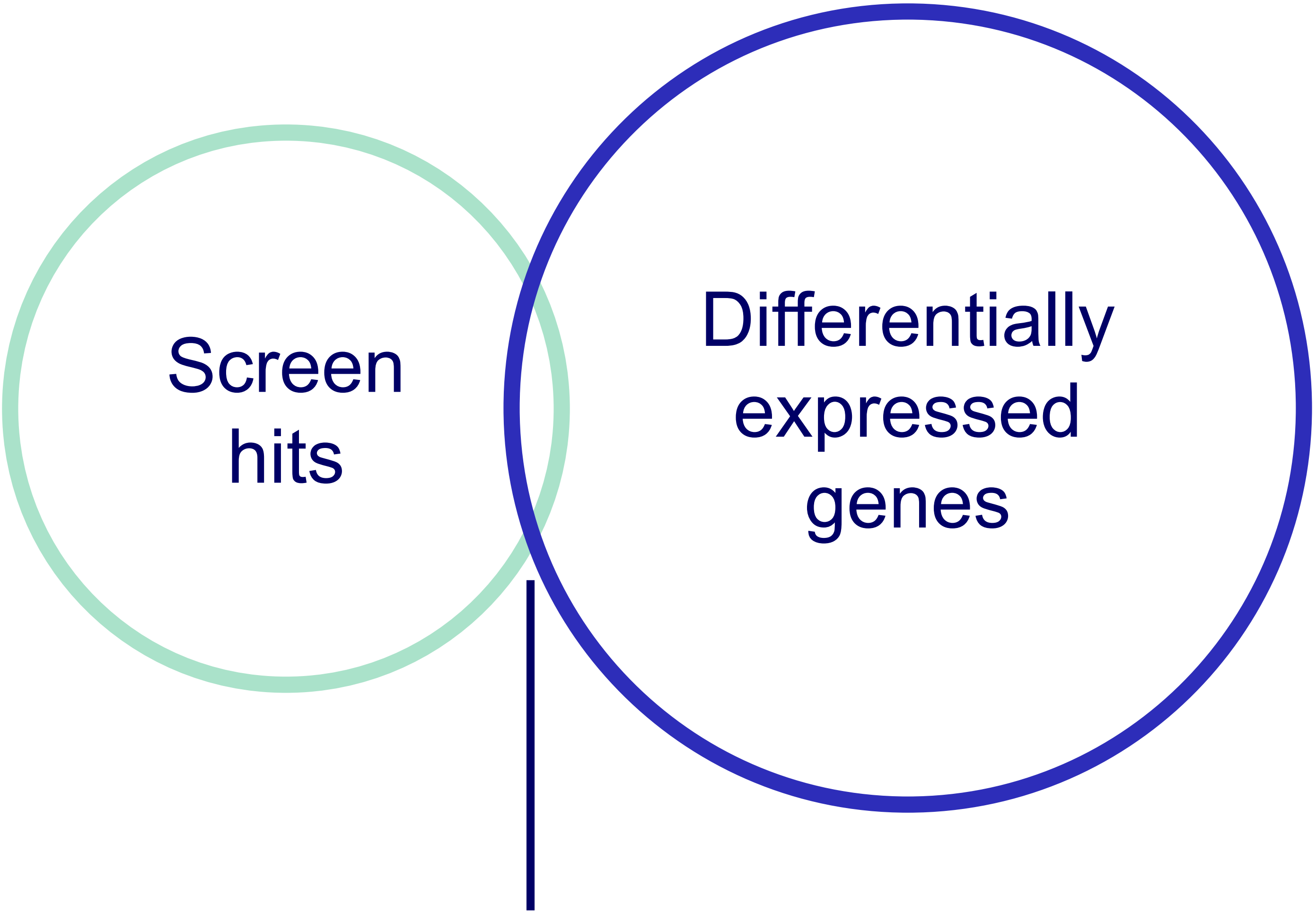
Types of screens

- Genetic screening
  - Test genes individually or in parallel
  - Knockout, knockdown (RNA interference), overexpression, CRISPR/Cas genome editing
- Chemical screening
  - Which genes are affected by a stimulus?

Differentially expressed genes

- Compare mRNA transcript levels between control and treatment conditions
- Genes whose expression changes significantly are also involved in the cellular process
- Alternatively, differential protein abundance or phosphorylation

Interpreting screens

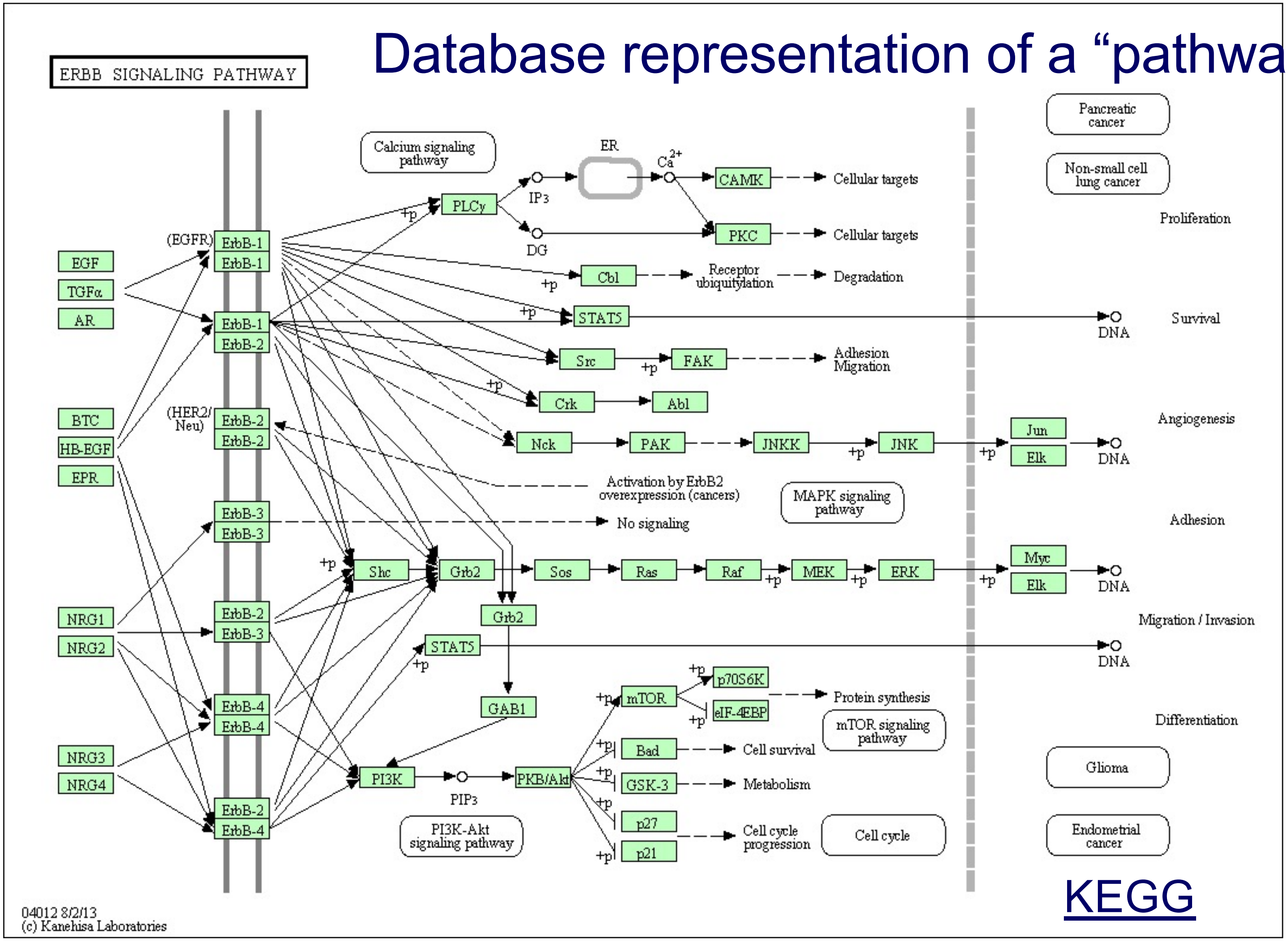


Screen hits

Differentially expressed genes

Very few genes detected in both

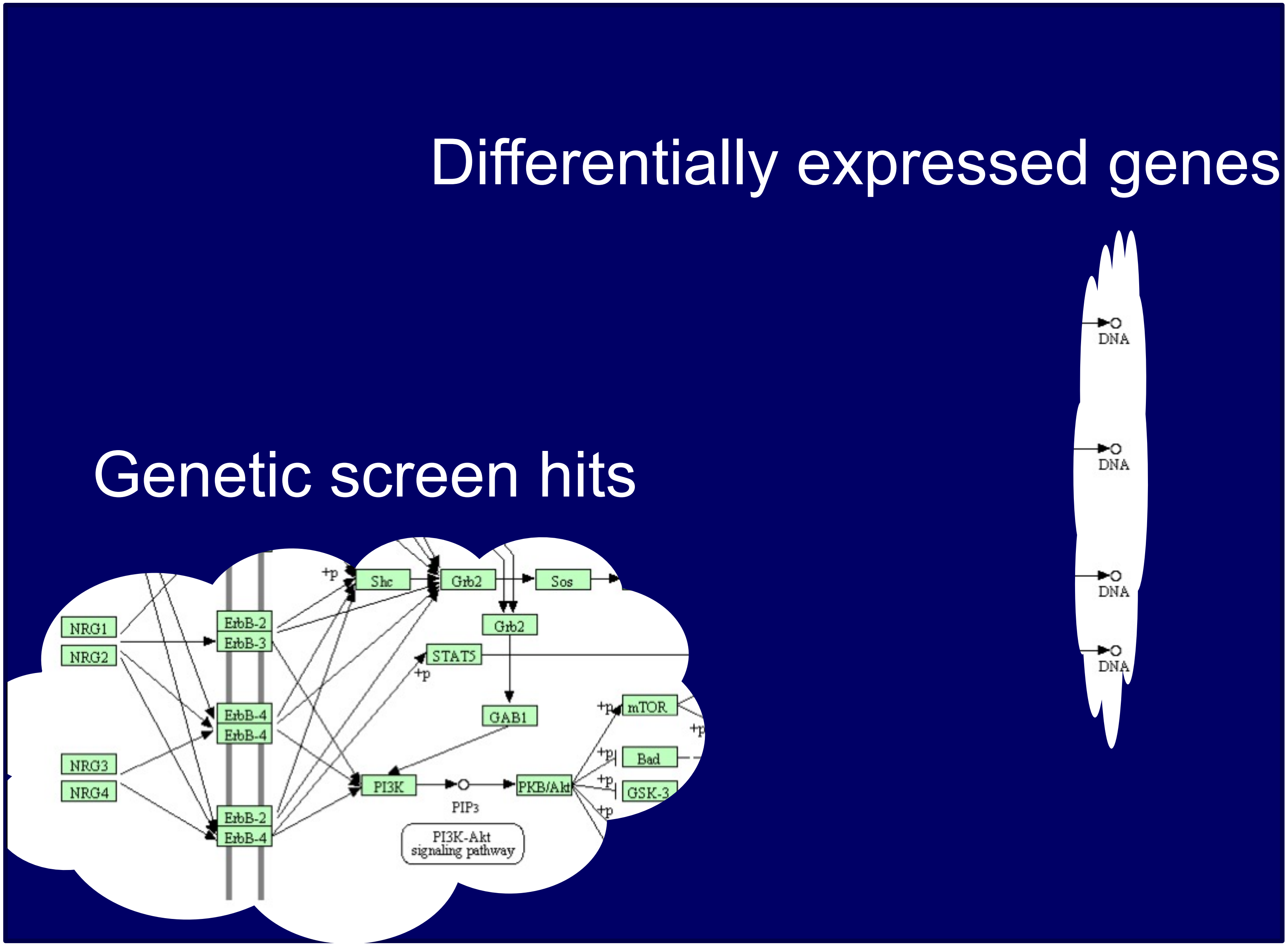
Assays reveal different parts of a cellular process



Database representation of a “pathway”

KEGG

Assays reveal different parts of a cellular process

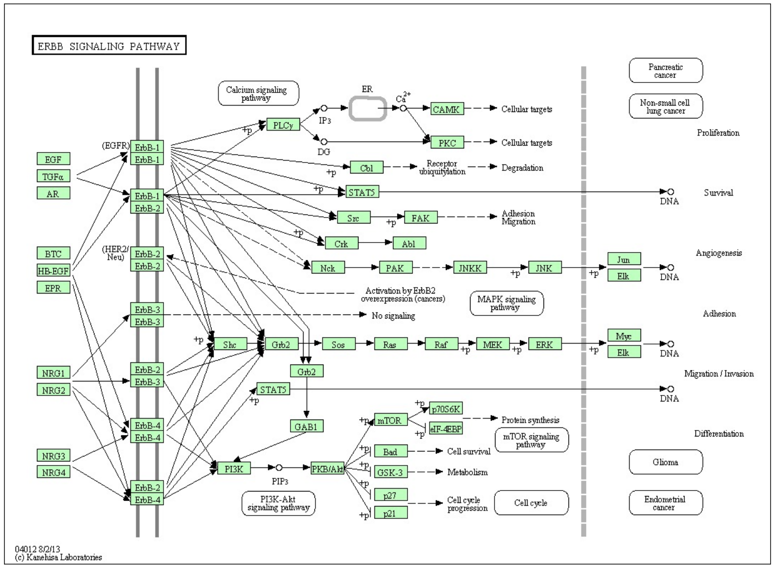


Differentially expressed genes

Genetic screen hits

Pathways connect the disjoint gene lists

- Can’t rely on pathway databases
- High-quality, low coverage

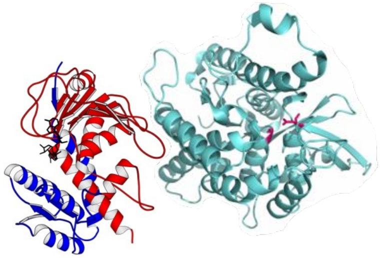


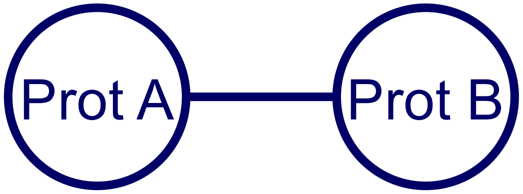
- Instead learn condition-specific pathways computationally
- Combine data with generic physical interaction networks

Physical interactions

- Protein-protein interactions (PPI)

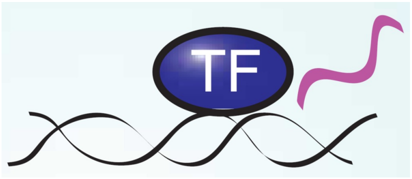
Appling Graz

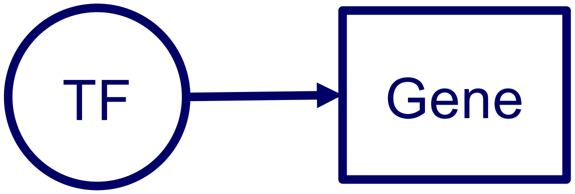




- Metabolic
- Protein-DNA (transcription factor-gene)

Yeger-Lotem2009

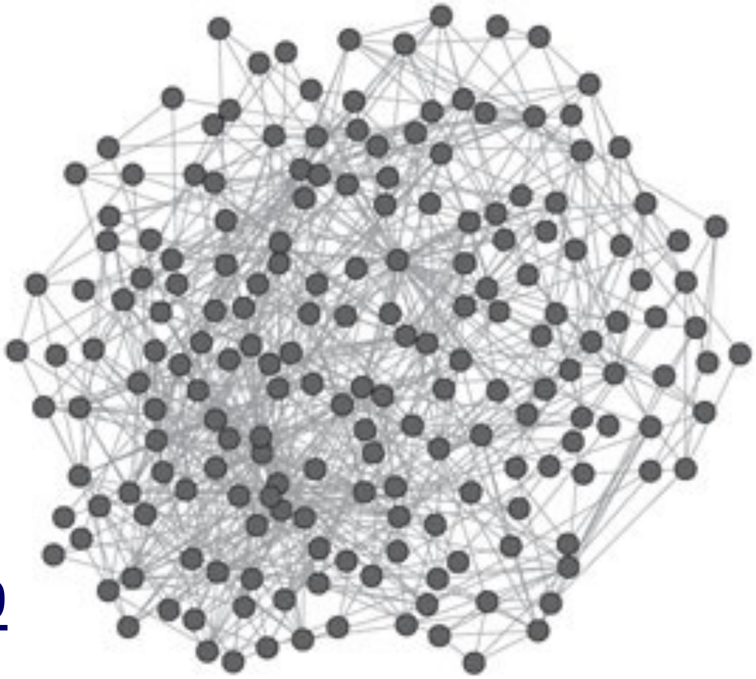




- Genes and proteins are different node types

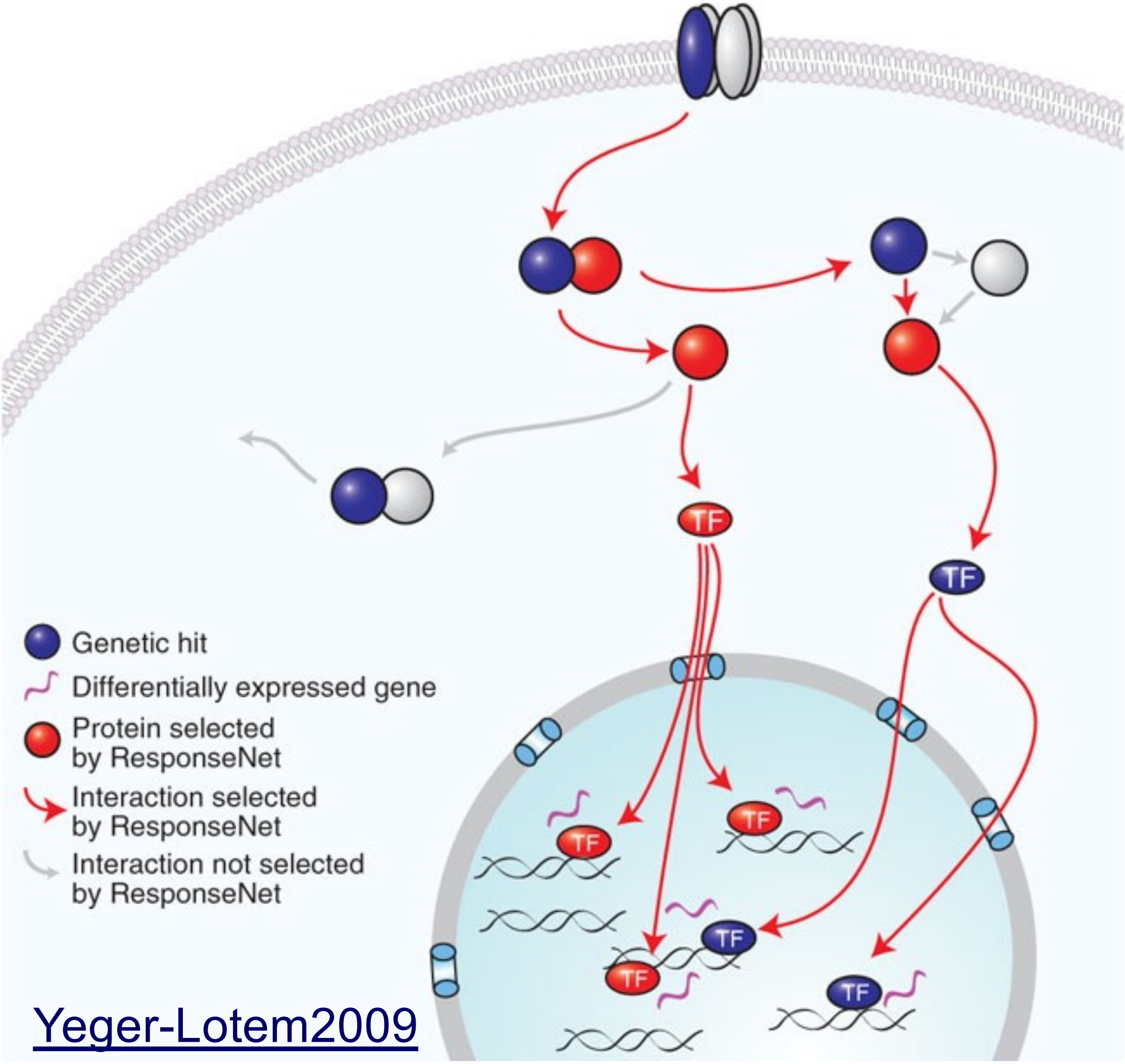
Hairball networks

- Networks are highly connected
- Can’t use naïve strategy to connect screen hits and differentially expressed genes



Yeger-Lotem2009

Identify connections within an interaction network

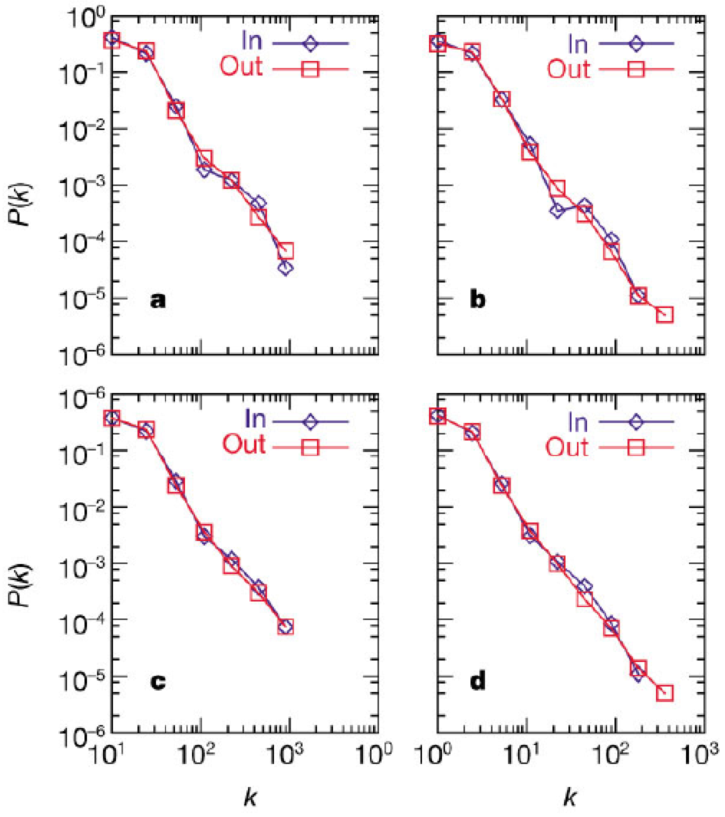

TF
TF
TF
TF
TF
TF
TF
Genetic hit
Differentially expressed gene
Protein selected by ResponseNet
Interaction selected by ResponseNet
Interaction not selected by ResponseNet

Yeger-Lotem2009

Biological Network Properties

- Degree: number of neighbors of a node
- Power law degree distribution
  - Most nodes have low degrees
  - Few highly connected nodes (hubs)
- Robust to random attacks
  - e.g., structure resilient to mutations
  - Mutations in hubs can damage the network
- Modular organization
  - High clustering coefficient (short paths)
  - Efficient signal propagation

Power law degree distribution



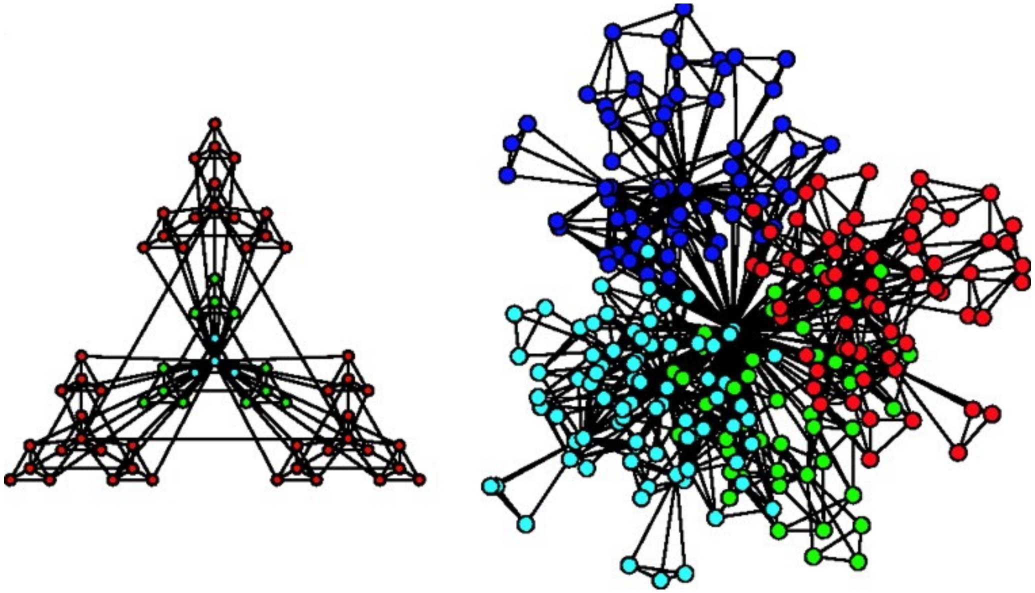
a) A. fulgidus (Archae)
b) Bacterium
c) C. elegans (Eukaryote),
d) averaged over 43 organisms

H. Jeong et al., Nature, 407 (2000)

- Probability of finding a highly connected node decreases exponentially with *K*

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

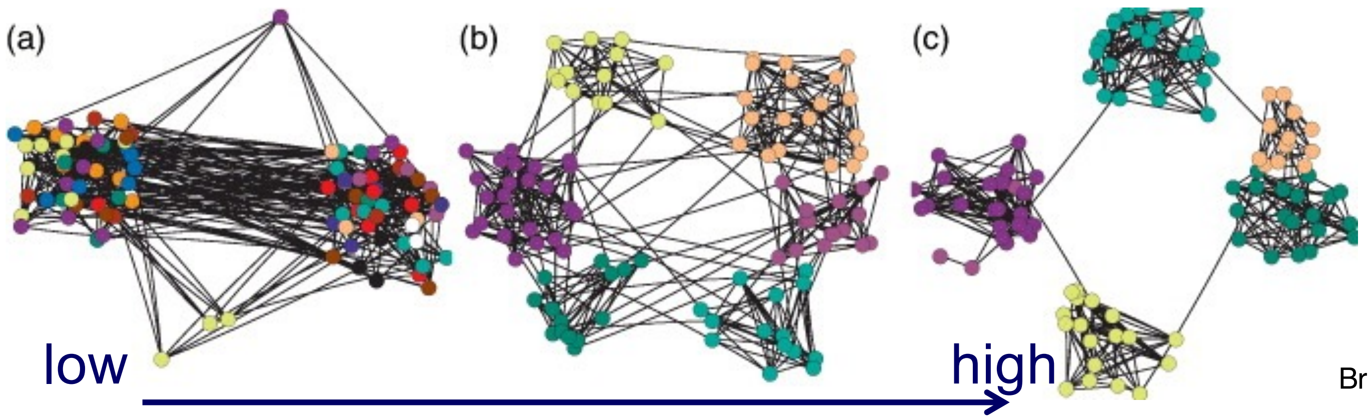
Modularity



E. Ravasz et al., Science 297, 1551 -1555 (2002)

- Small highly connected cohesive clusters that combine to form larger units
- Communication between clusters through hubs
- Hierarchical modularity overlaps with known metabolic functions

Measurement of Modularity



Brede, Europhysics Letters, 2010.

Modularity Q: measurement on strength of network division

$$Q = \frac{1}{2m}\sum_{i,j}\left(W_{ij} - \frac{k_i k_j}{2m}\right)\delta_{\sigma_i \sigma_j}$$

sum over nodes within a group (module)

normalization
m: total number of edges

edge weight between nodes i and j

$\frac{k_i k_j}{2m} = p_{ij}$=expected edge weight that would go between i and j

Clustering goal: assign each node a module to maximize “modularity” as an objective function (module is a group of highly connected nodes)

Newman, PNAS, 2006.

Clustering coefficient

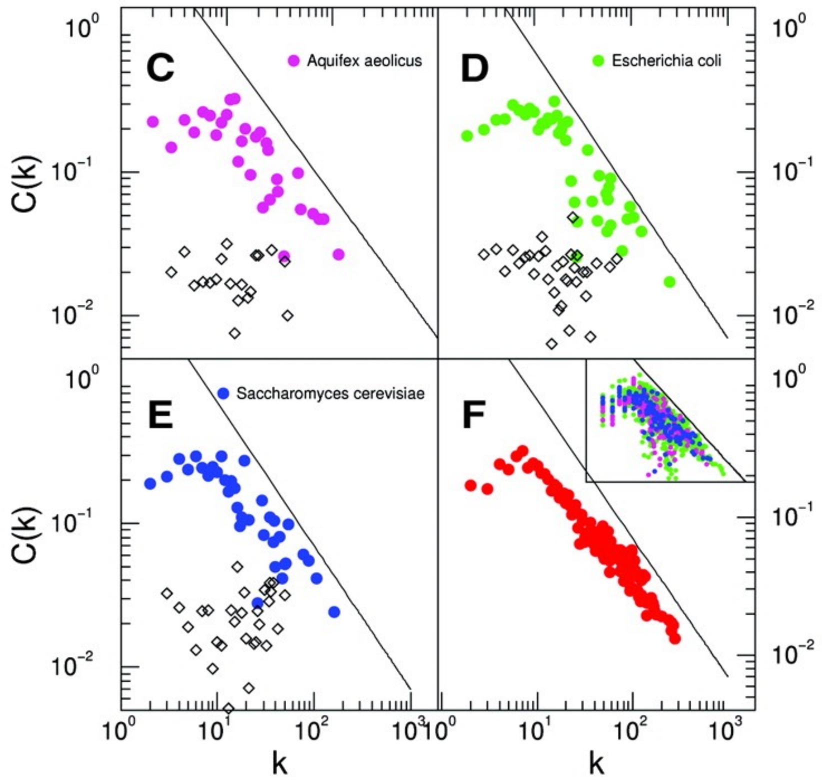
Measures the average probability that two neighbors of a node are connected

$$C_I = \frac{n_I}{\binom{k}{2}} = \frac{2n_I}{k \cdot (k-1)}$$

n_I: # edges between node *I*’s neighbors

k: # of neighbors of *I*

Clustering coefficient

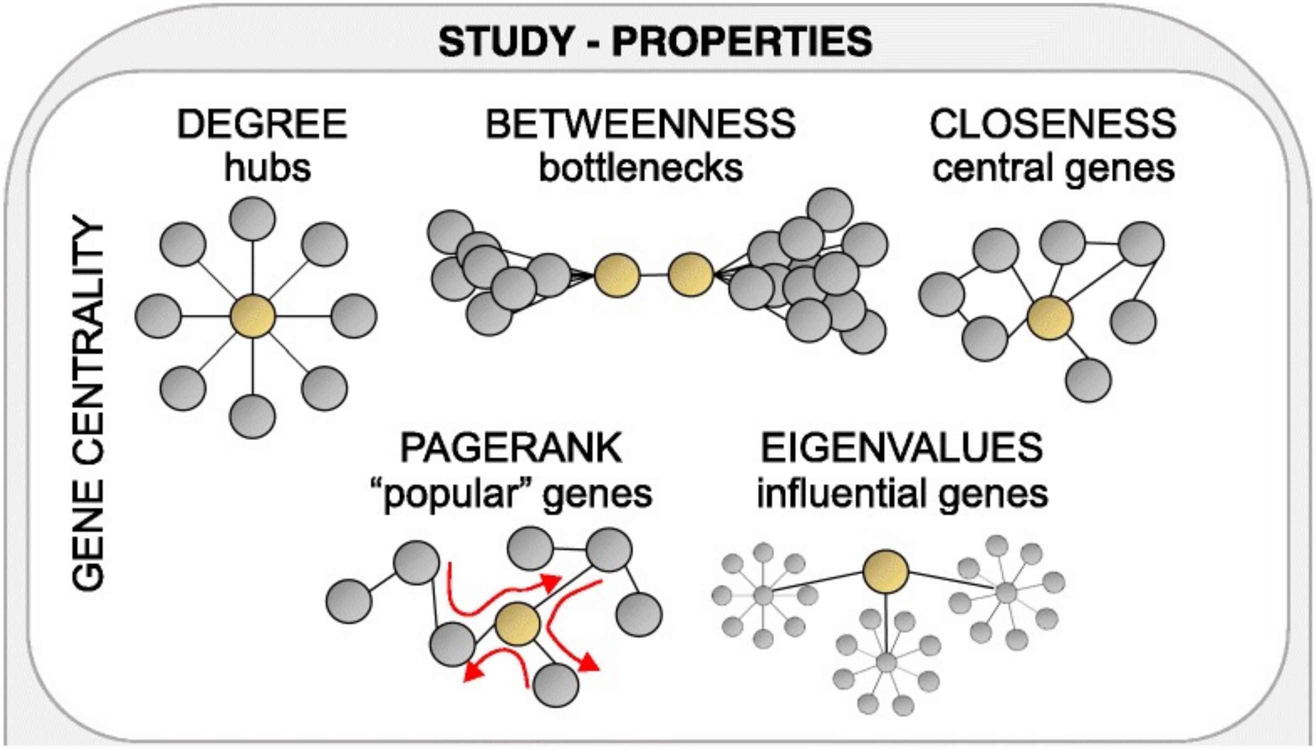


- High degree nodes -> low clustering coefficient CC
- Network’s modularity -> CC averaged over all nodes
- Metabolic networks have high intrinsic modularity

E. Ravasz et al., Science 297, 1551 -1555 (2002)

Network centralities

Topological importance of a node



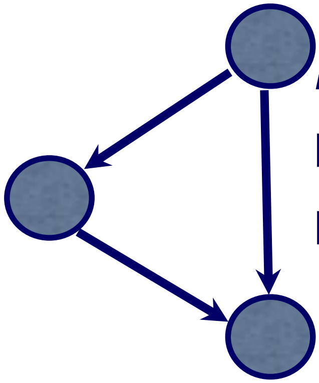
G. Iacono et al., Genome Biology 20 (2019)

Network problems

- Network inference
  - Infer network structure
- Motif finding
  - Identify common subgraph topologies
- Pathway or module detection
  - Identify subgraphs of genes that perform the same function or active in same condition
- Network comparison, alignment, querying
- Conserved modules
  - Identify modules that are shared in networks of multiple species/conditions

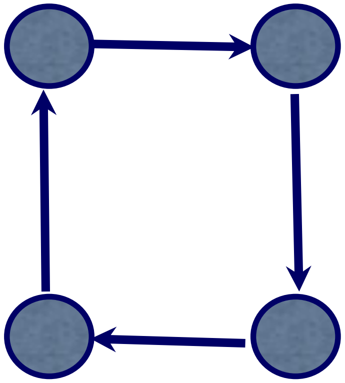
Network motifs

- Problem: Find subgraph topologies that are statistically more frequent than expected
- Brute force approach
  - Count all topologies of subgraphs of size m
  - Randomize graph (retain degree distribution) and count again
  - Output topologies that are over/under represented

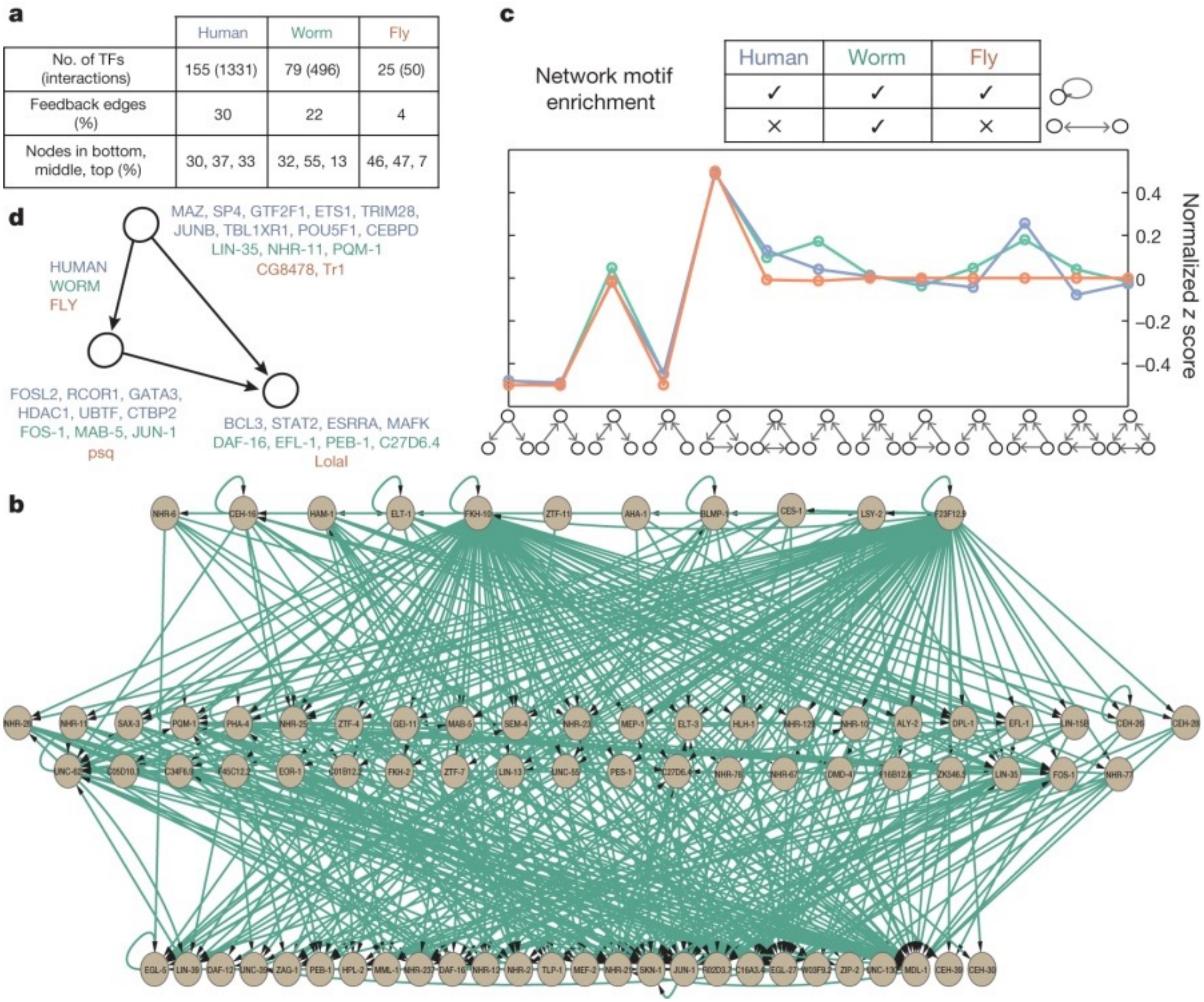


Feed-forward loop: over-represented in regulatory networks

not very common



Gene regulatory network motifs



a

	Human	Worm	Fly
No. of TFs (interactions)	155 (1331)	79 (496)	25 (50)
Feedback edges (%)	30	22	4
Nodes in bottom, middle, top (%)	30, 37, 33	32, 55, 13	46, 47, 7

c

Network motif enrichment	Human	Worm	Fly
	✓	✓	✓
	×	✓	×

AP Boyle *et al.* *Nature* **512** , 453-456 (2014) doi:10.1038/nature13668

Network modules

- Modules: dense (highly-connected) subgraphs (e.g., large cliques or partially incomplete cliques)
- Problem: Identify the component modules of a network
- Difficulty: definition of module is not precise
  - Hierarchical networks have modules at multiple scales
  - At what scale to define modules?

How to define a computational "pathway"

- **Given:**
  - Partially directed network of known physical interactions (e.g. PPI, kinase-substrate, TF-gene)
  - Scores on source nodes
  - Scores on target nodes

- **Do:**
  - Return directed paths in the network connecting sources to targets

Network flow problem

- Finding an optimal route by minimizing transportation costs from LA to NYC
  - $c_{i,j}$, the cost between City *i* and City *j*
  - $f_{i,j}$ = 1 if in route, = 0 if not
  - $\text{argmin}_f \sum_{i,j} c_{i,j} * f_{i,j}$ s.t. constraints

NYC
LA
INTERSTATE

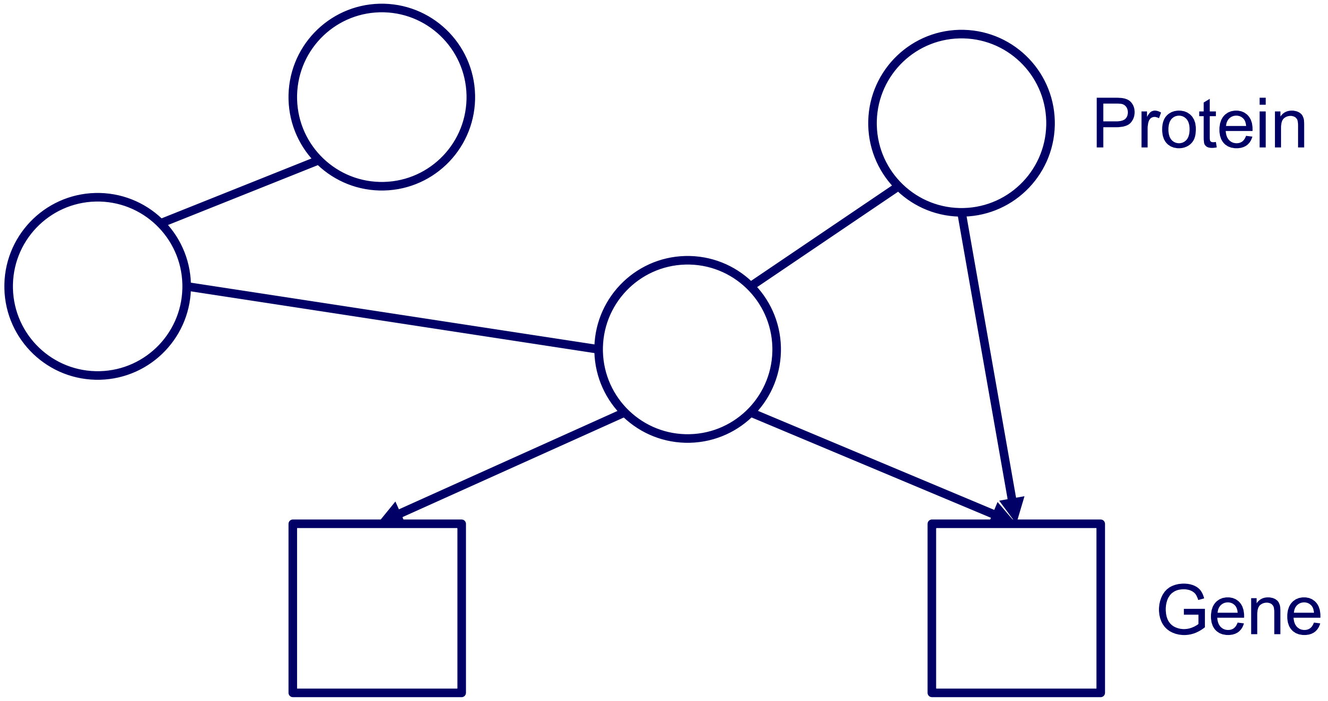
https://www.visualcapitalist.com/u-s-interstate-highways-transit-map/

ResponseNet optimization goals

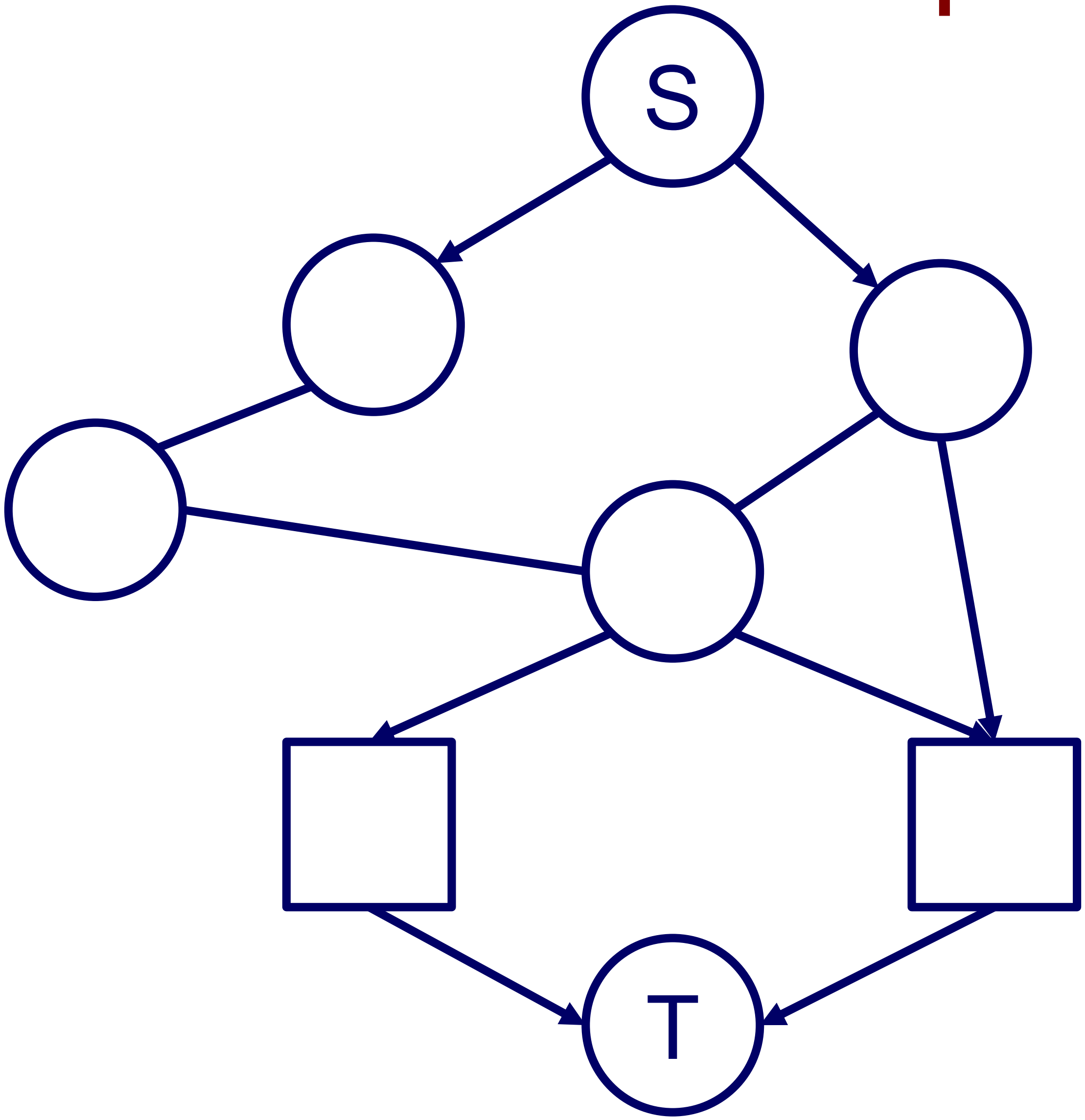
- Connect screen hits and differentially expressed genes
- Recover sparse connections
- Identify intermediate proteins missed by the screens
- Prefer high-confidence interactions

- Minimum cost flow formulation can meet these objectives

Construct the interaction network

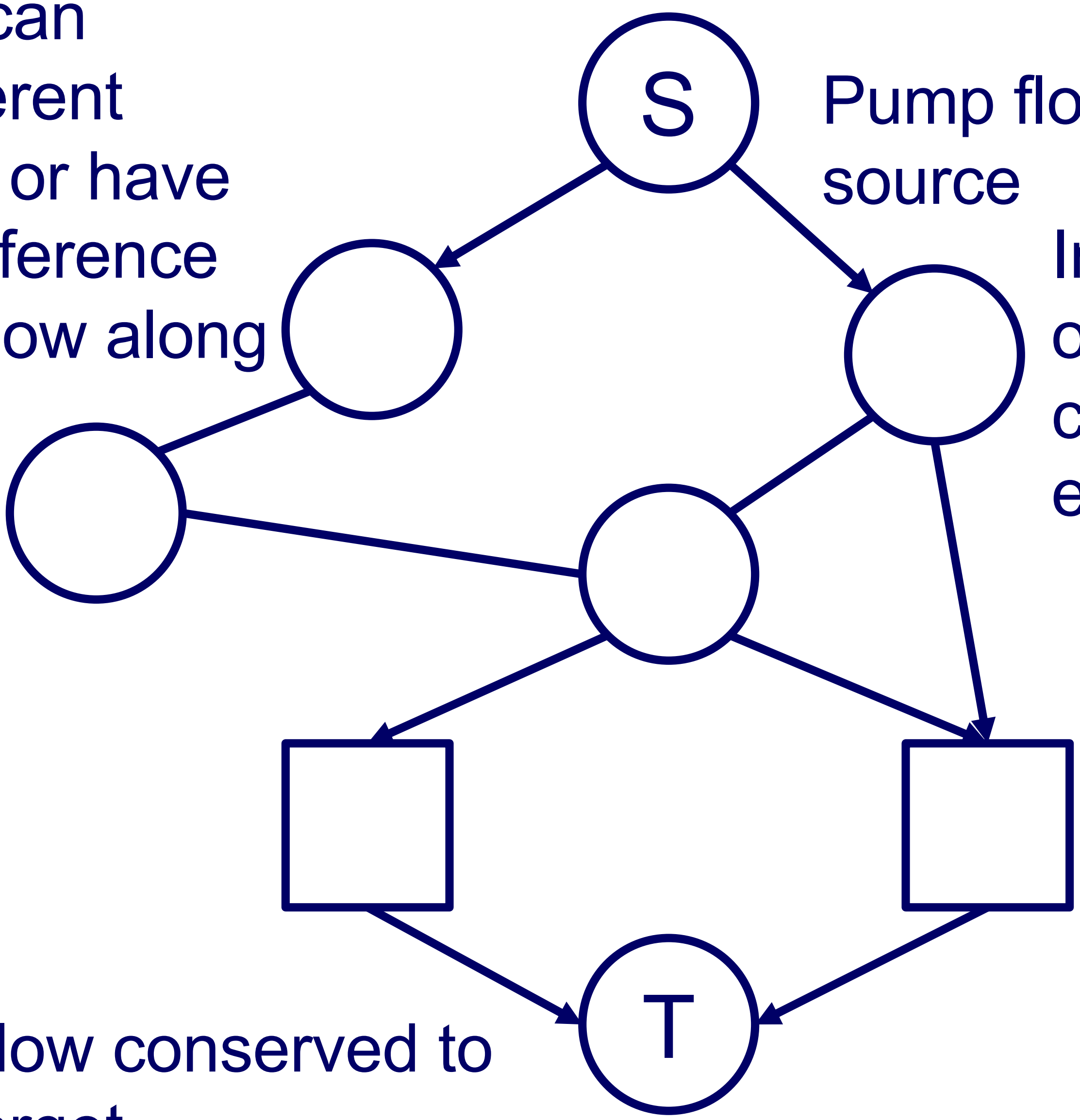


Transform to a flow problem



Max flow on graphs

Each edge can tolerate different level of flow or have different preference of sending flow along that edge



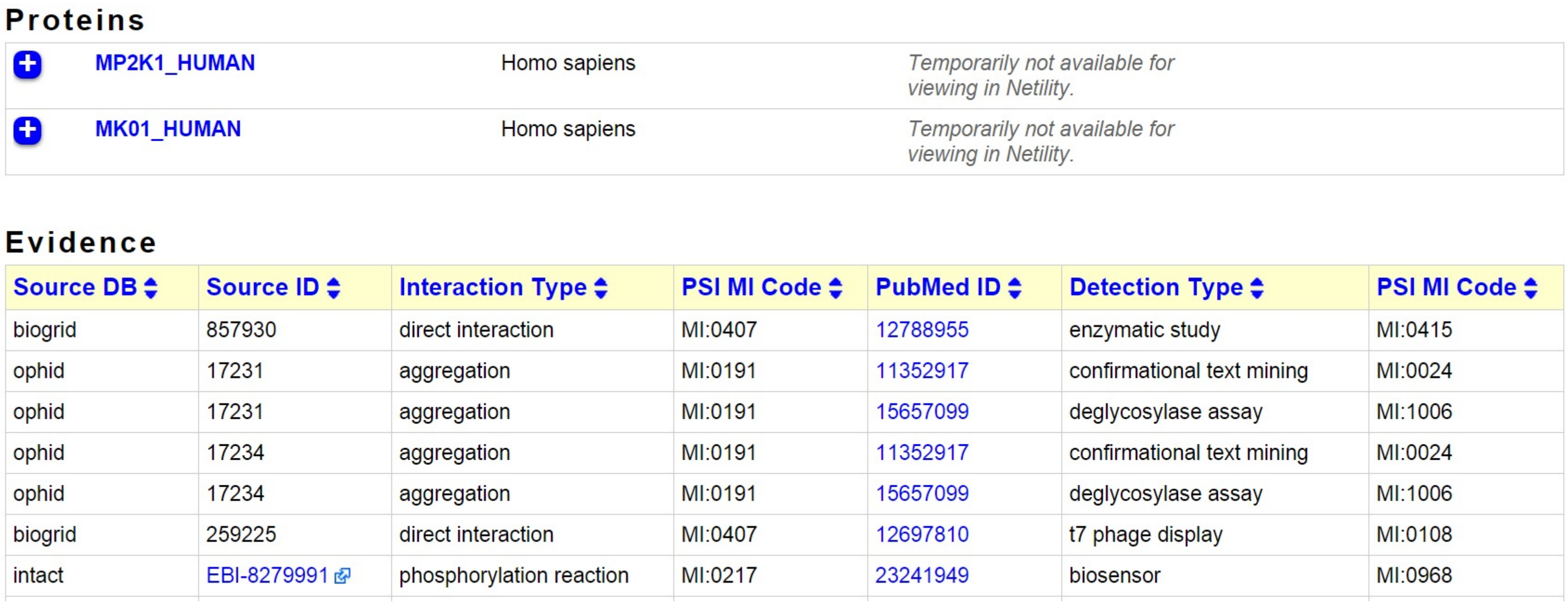
Pump flow from source

Incoming and outgoing flow conserved at each node

Flow conserved to target

Weighting interactions

- Probability-like confidence of the interaction



Proteins

+	MP2K1_HUMAN	Homo sapiens	*Temporarily not available for viewing in Netility.*
+	MK01_HUMAN	Homo sapiens	*Temporarily not available for viewing in Netility.*

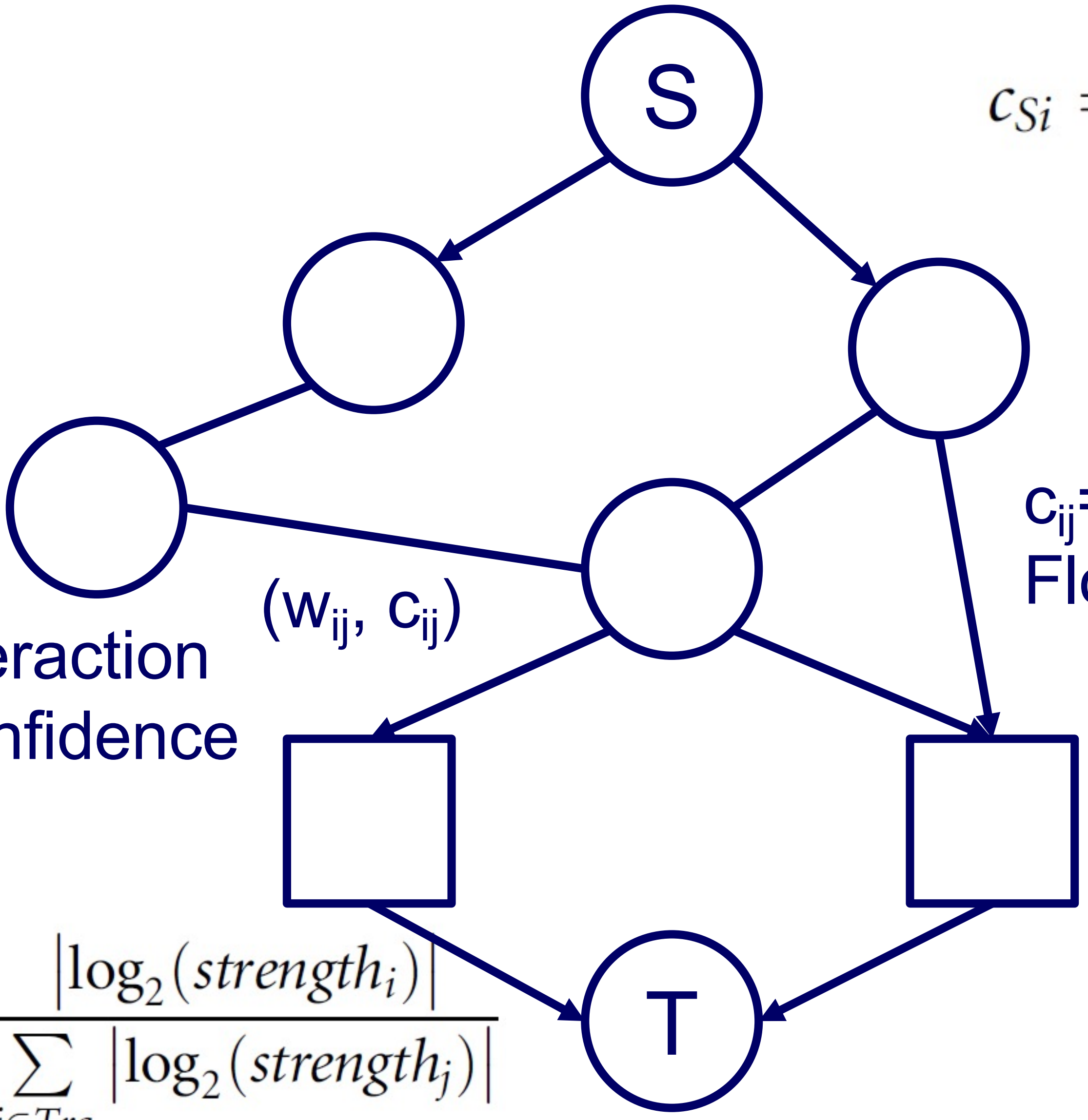
Evidence

Source DB	Source ID	Interaction Type	PSI MI Code	PubMed ID	Detection Type	PSI MI Code
biogrid	857930	direct interaction	MI:0407	12788955	enzymatic study	MI:0415
ophid	17231	aggregation	MI:0191	11352917	confirmational text mining	MI:0024
ophid	17231	aggregation	MI:0191	15657099	deglycosylase assay	MI:1006
ophid	17234	aggregation	MI:0191	11352917	confirmational text mining	MI:0024
ophid	17234	aggregation	MI:0191	15657099	deglycosylase assay	MI:1006
biogrid	259225	direct interaction	MI:0407	12697810	t7 phage display	MI:0108
intact	EBI-8279991	phosphorylation reaction	MI:0217	23241949	biosensor	MI:0968

iRefWeb

- Example evidence: edge score of 1.0
- 16 distinct publications supporting the edge

Weights and capacities on edges



$$c_{Si} = \frac{|strength_i|}{\sum_{j \in Gen} |strength_j|}$$

(w_{ij}, c_{ij})

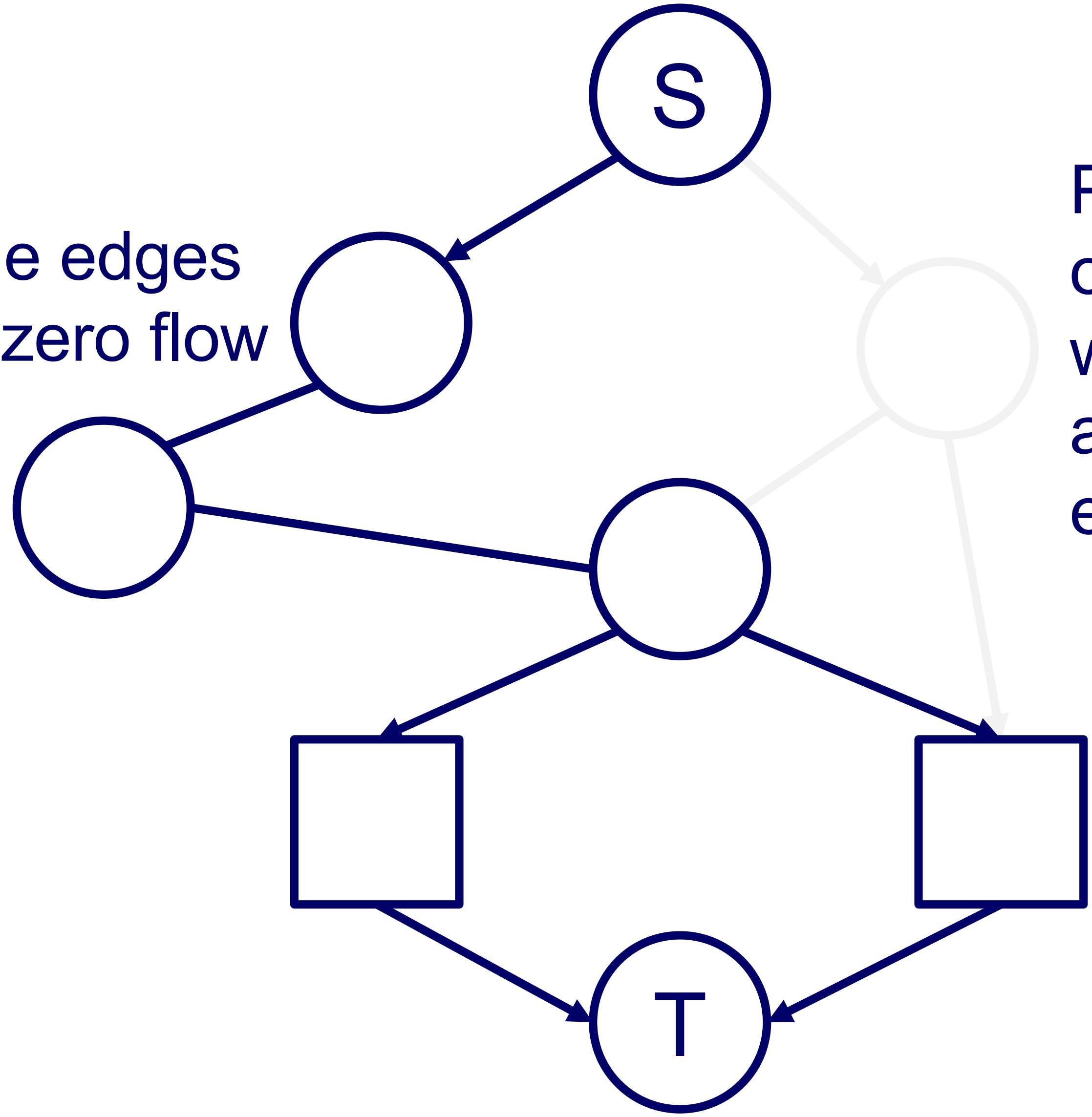
c_{ij}= 1
Flow capacity

w_{ij} from interaction
network confidence

$$c_{iT} = \frac{|\log_2(strength_i)|}{\sum_{j \in Tra} |\log_2(strength_j)|}$$

Find the minimum cost flow

Return the edges with non-zero flow



Prefer no flow on the low-weight edges if alternative paths exist

Formal minimum cost flow

$$\min_{f}\left(\left(\sum_{i\in V',j\in V'} -\log(w_{ij}) * f_{ij}\right) - \left(\gamma * \sum_{i\in Gen} f_{Si}\right)\right)$$

Positive flow on an edge incurs a cost

Cost is greater for low-weight edges

Flow on an edge

Parameter controlling the amount of flow from the source

Formal minimum cost flow

$$\min_f((\sum_{i\in V',j\in V'} -\log(w_{ij}) * f_{ij}) - (\gamma * \sum_{i\in Gen} f_{Si}))$$

Subject to:

$$\sum_{j\in V'} f_{ij} - \sum_{j\in V'} f_{ji} = 0 \quad \forall i \in V' - \{S, T\}$$

Flow coming in to a node equals flow leaving the node

Formal minimum cost flow

$$\min_{f}((\sum_{i\in V',j\in V'} -\log(w_{ij}) * f_{ij}) - (\gamma * \sum_{i\in Gen} f_{Si}))$$

Subject to:

$$\sum_{j\in V'} f_{ij} - \sum_{j\in V'} f_{ji} = 0 \quad \forall i \in V' - \{S, T\}$$

$$\sum_{i\in Gen} f_{Si} - \sum_{i\in Tra} f_{iT} = 0$$

Flow leaving the source equals flow entering the target

Formal minimum cost flow

$$\min_f((\sum_{i\in V',j\in V'} -\log(w_{ij}) * f_{ij}) - (\gamma * \sum_{i\in Gen} f_{Si}))$$

Subject to:

$$\sum_{j\in V'} f_{ij} - \sum_{j\in V'} f_{ji} = 0 \quad \forall i \in V' - \{S, T\}$$

$$\sum_{i\in Gen} f_{Si} - \sum_{i\in Tra} f_{iT} = 0$$

Flow is non-negative and does not exceed edge capacity

$$0 \leq f_{ij} \leq c_{ij} \quad \forall (i, j) \in E'$$

Formal minimum cost flow

$$\min_{f}((\sum_{i\in V',j\in V'} -\log(w_{ij}) * f_{ij}) - (\gamma * \sum_{i\in Gen} f_{Si}))$$

Subject to:

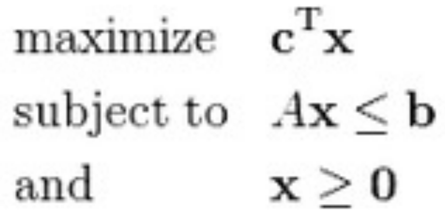
$$\sum_{j\in V'} f_{ij} - \sum_{j\in V'} f_{ji} = 0 \quad \forall i \in V' - \{S, T\}$$

$$\sum_{i\in Gen} f_{Si} - \sum_{i\in Tra} f_{iT} = 0$$

$$0 \leq f_{ij} \leq c_{ij} \quad \forall (i, j) \in E'$$

Linear programming

- Optimization problem is a linear program
- Canonical form

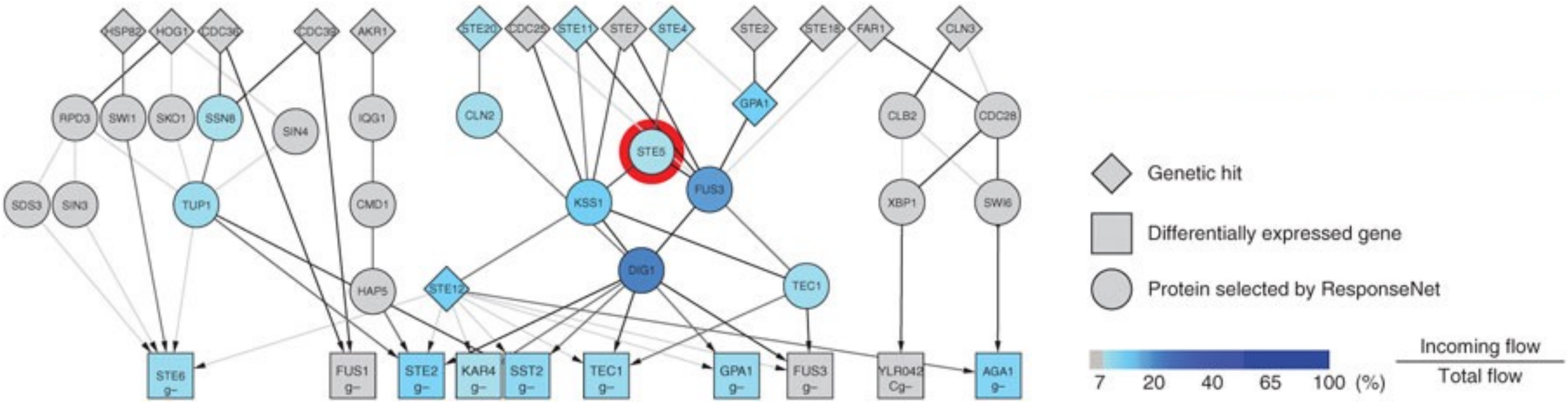


$$\begin{array}{ll} \text{maximize} & \mathbf{c}^{\mathrm{T}}\mathbf{x} \\ \text{subject to} & A\mathbf{x} \leq \mathbf{b} \\ \text{and} & \mathbf{x} \geq \mathbf{0} \end{array}$$

Wikipedia

- Polynomial time complexity
- Many off-the-shelf solvers
- Practical Optimization: A Gentle Introduction
  – Introduction to linear programming
  – Simplex method
  – Network flow

ResponseNet pathways



- Identifies pathway members that are neither hits nor differentially expressed
- Ste5 recovered when *STE5* deletion is the perturbation

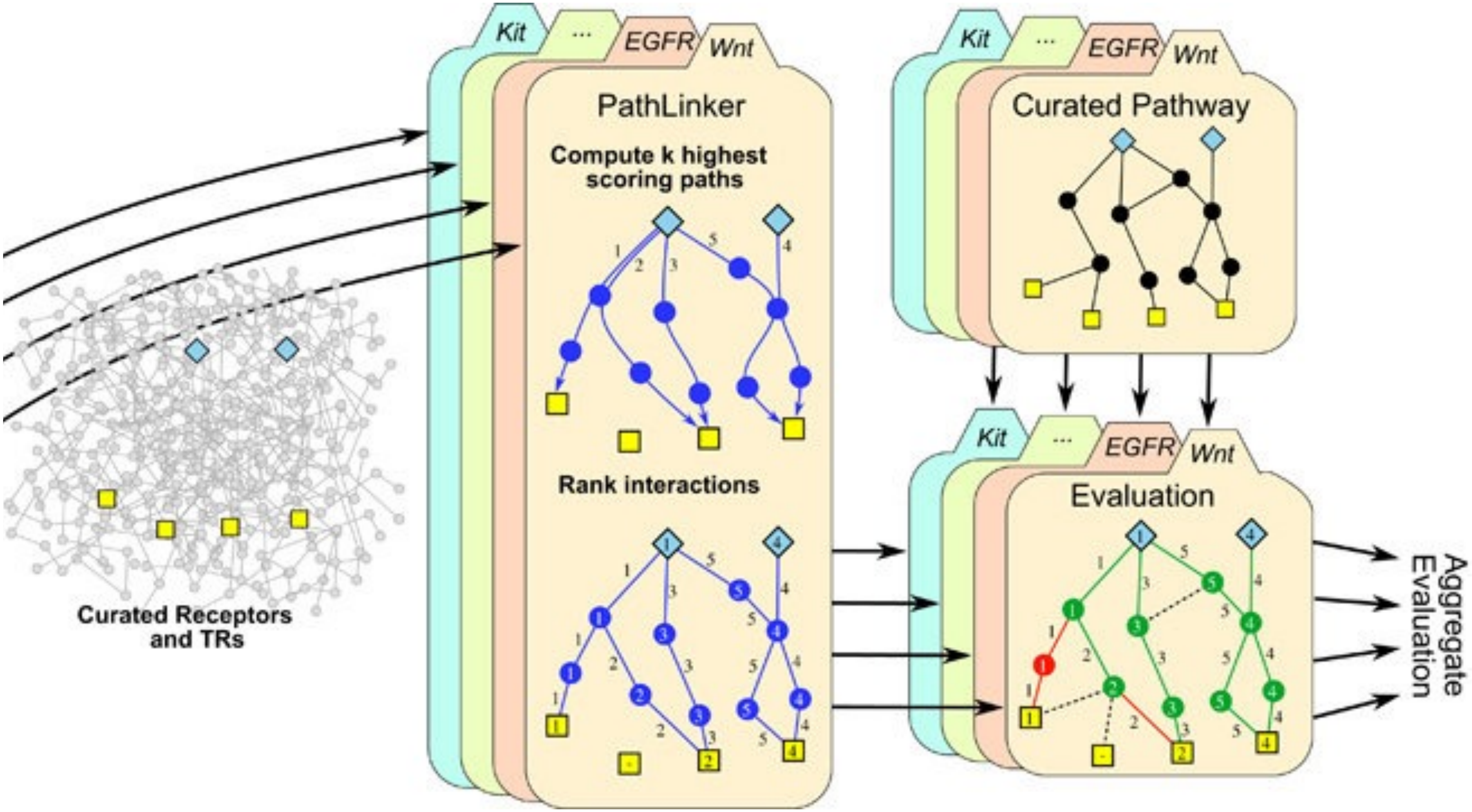
ResponseNet summary

- Advantages
  - Computationally efficient
  - Integrates multiple types of data
  - Incorporates interaction confidence
  - Identifies biologically plausible networks
- Disadvantages
  - Direction of flow is not biologically meaningful
  - Path length not considered
  - Requires sources and targets
  - Dependent on completeness and quality of input network

Evaluating pathway predictions

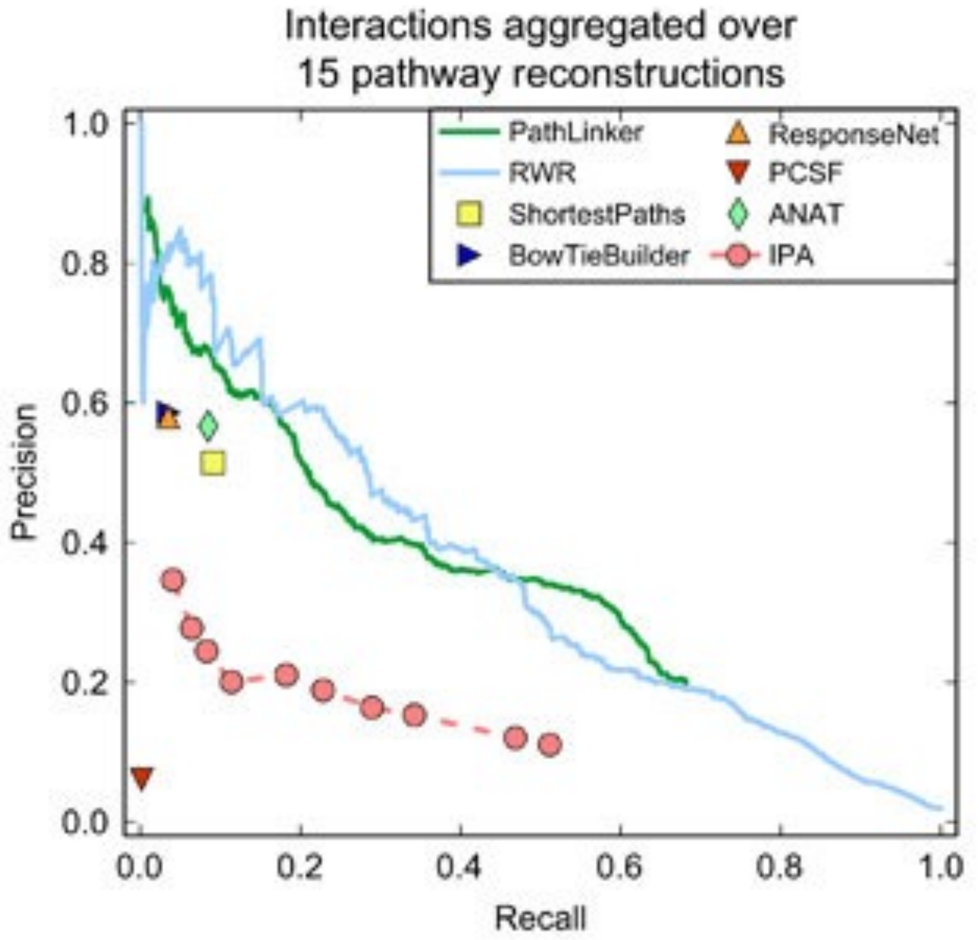
- Unlike PIQ, we don’t have a complete gold standard available for evaluation
- Can simulate “gold standard” pathways from a network
- Compare relative performance of multiple methods on independent data

Evaluating pathway predictions



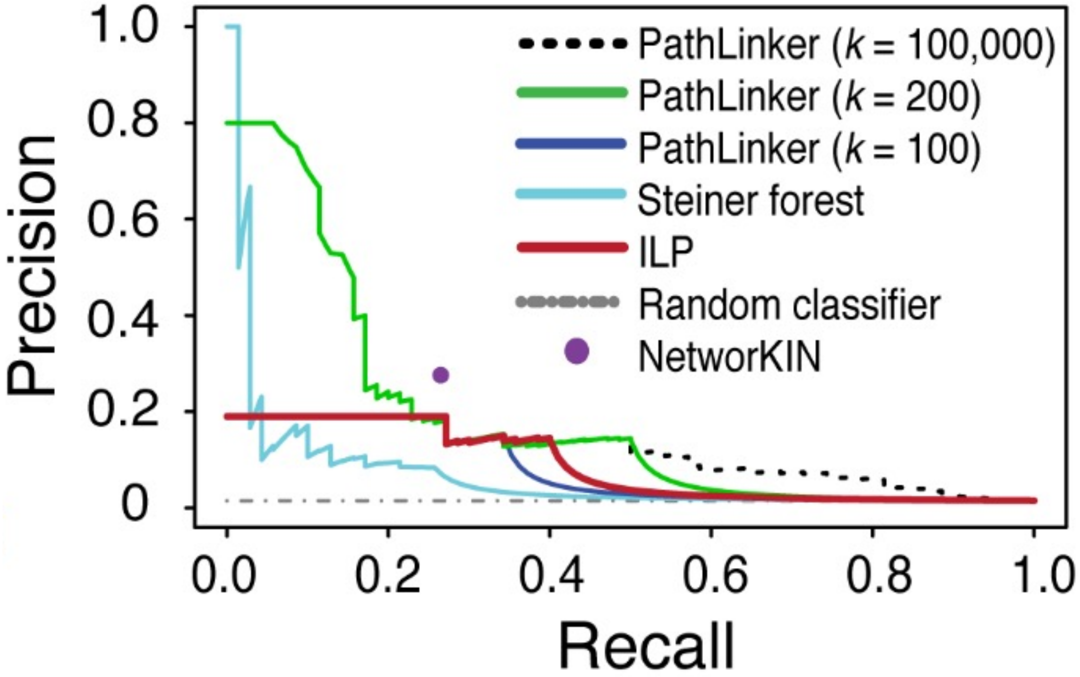
[Ritz2016](https://www.nature.com/articles/npjsba20162.pdf) https://www.nature.com/articles/npjsba20162.pdf

Evaluating pathway predictions



Ritz2016

Evaluating pathway predictions

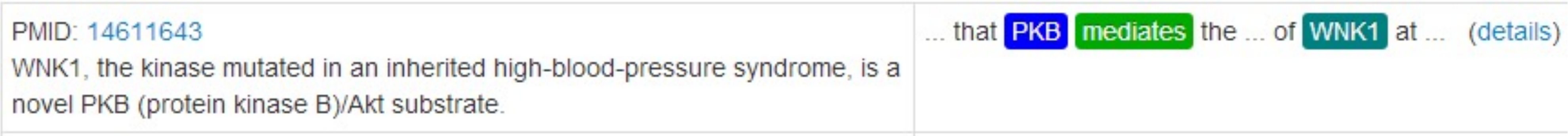


MacGilvray2018

- PR curves can evaluate node or edge recovery but not the global pathway structure

Evaluation beyond pathway databases

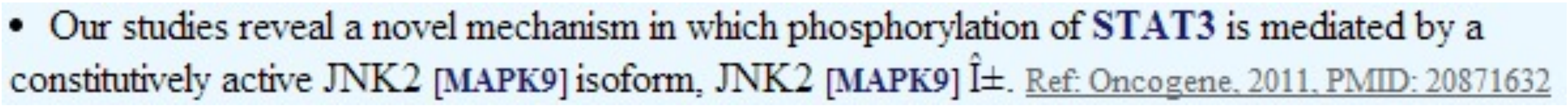
- Natural language processing can also help semi-automated evaluation
  - Literome



PMID: 14611643
WNK1, the kinase mutated in an inherited high-blood-pressure syndrome, is a novel PKB (protein kinase B)/Akt substrate.

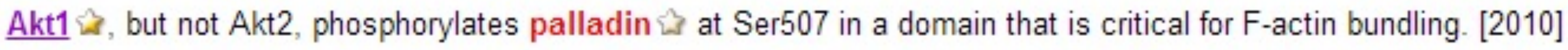
... that PKB mediates the ... of WNK1 at ... (details)

  - Chilibot



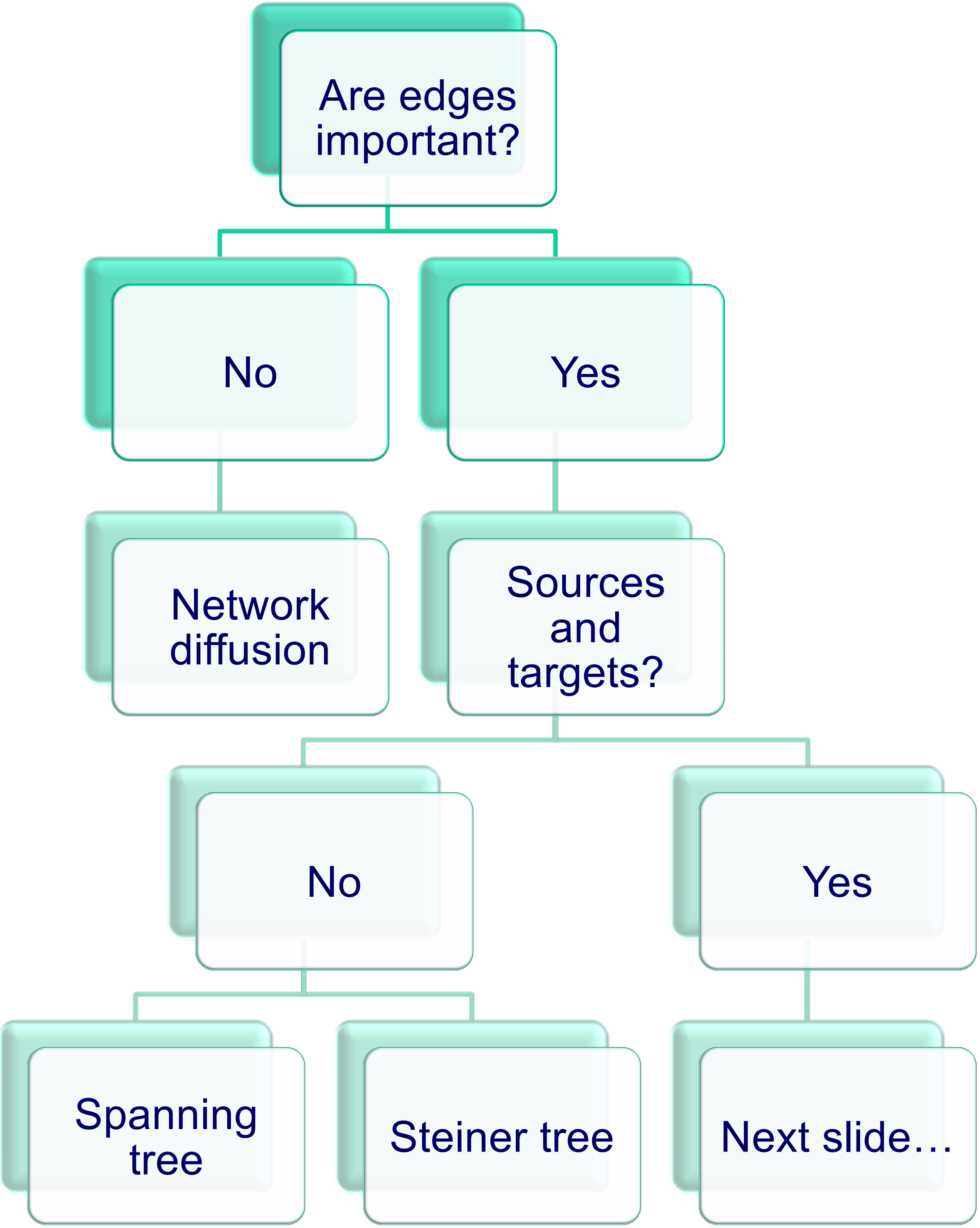
- Our studies reveal a novel mechanism in which phosphorylation of **STAT3** is mediated by a constitutively active JNK2 [**MAPK9**] isoform, JNK2 [**MAPK9**] Î±. Ref: Oncogene. 2011. PMID: 20871632

  - iHOP

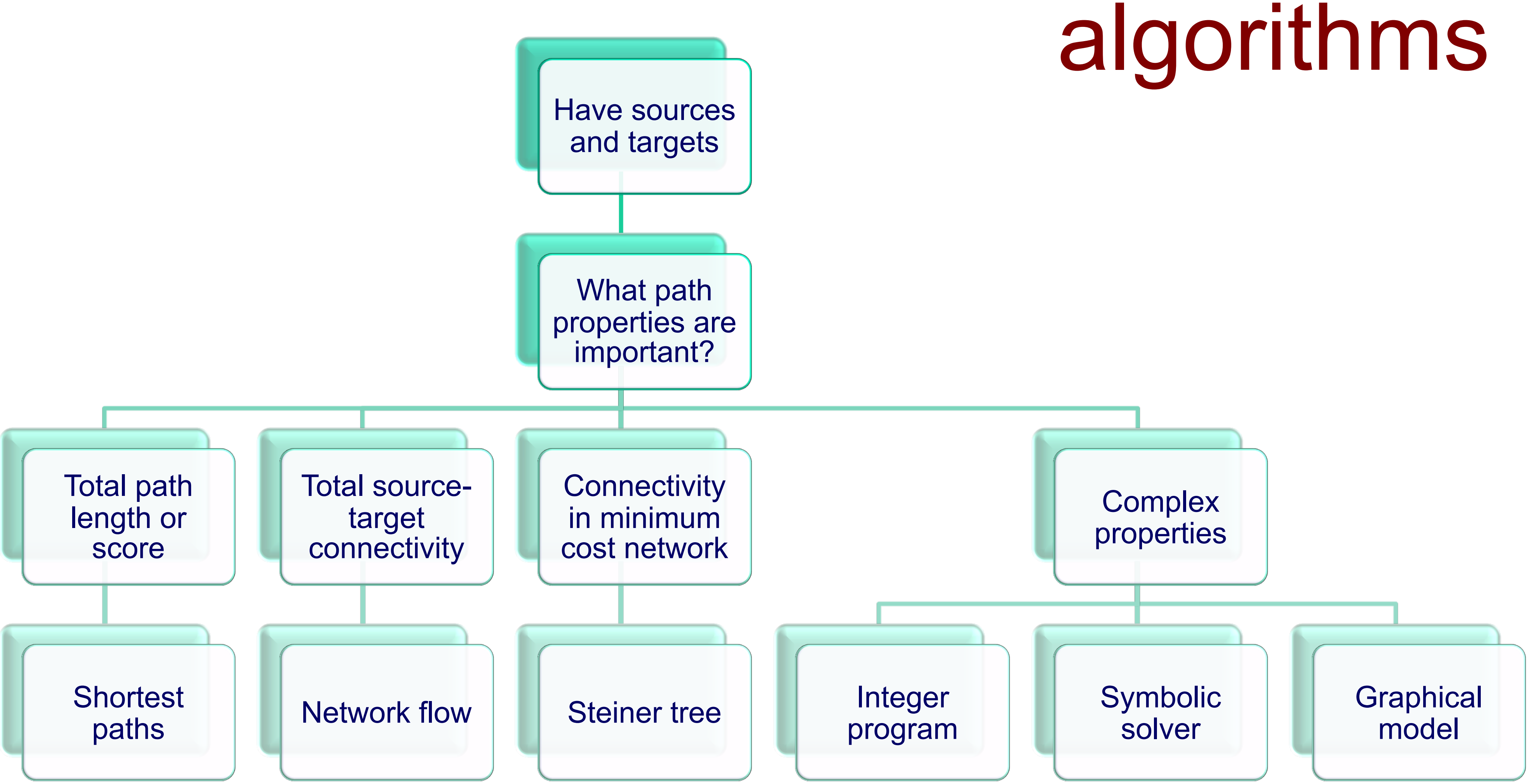


Akt1, but not Akt2, phosphorylates **palladin** at Ser507 in a domain that is critical for F-actin bundling. [2010]

Classes of pathway prediction algorithms



Classes of pathway prediction algorithms


Have sources and targets
What path properties are important?
Total path length or score
Total source-target connectivity
Connectivity in minimum cost network
Complex properties
Shortest paths
Network flow
Steiner tree
Integer program
Symbolic solver
Graphical model

Alternative pathway identification algorithms

- k-shortest paths
  - Ruths2007
  - Shih2012
- Random walks / network diffusion / circuits
  - Tu2006
  - eQTL electrical diagrams (eQED)
  - HotNet
- Integer programs
  - Signaling-regulatory Pathway INferencE (SPINE)
  - Chasman2014

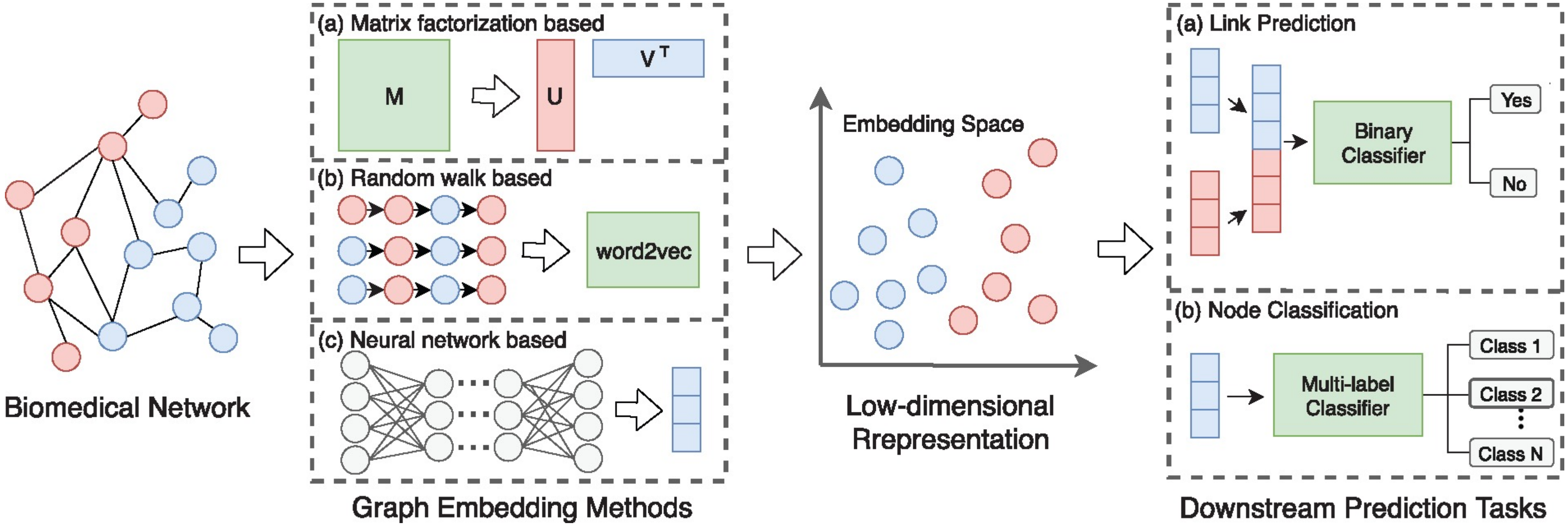
Alternative pathway identification algorithms

- Path-based objectives
  - Physical Network Models (PNM)
  - Maximum Edge Orientation (MEO)
  - Signaling and Dynamic Regulatory Events Miner (SDREM)
- Steiner tree
  - Prize-collecting Steiner forest (PCSF)
  - Belief propagation approximation (msgsteiner)
  - Omics Integrator implementation
- Hybrid approaches
  - PathLinker: random walk + shortest paths
  - ANAT: shortest paths + Steiner tree

Recent developments in pathway discovery

- Multi-task learning: jointly model several related biological conditions
  - ResponseNet extension: SAMNet
  - Steiner forest extension: Multi-PCSF
  - SDREM extension: MT-SDREM
- Temporal data
  - ResponseNet extension: TimeXNet
  - Steiner forest extension and ST-Steiner
  - Temporal Pathway Synthesizer

Graph embedding for biological networks



Bioinformatics, Volume 36, Issue 4, 15 February 2020, Pages 1241–1251, https://doi.org/10.1093/bioinformatics/btz718

Condition-specific genes/proteins used as input

- Genetic screen hits (as causes or effects)
- Differentially expressed genes
- Transcription factors inferred from gene expression
- Proteomic changes (protein abundance or post-translational modifications)
- Kinases inferred from phosphorylation
- Genetic variants or DNA mutations
- Enzymes regulating metabolites
- Receptors or sensory proteins
- Protein interaction partners
- Pathway databases or other prior knowledge