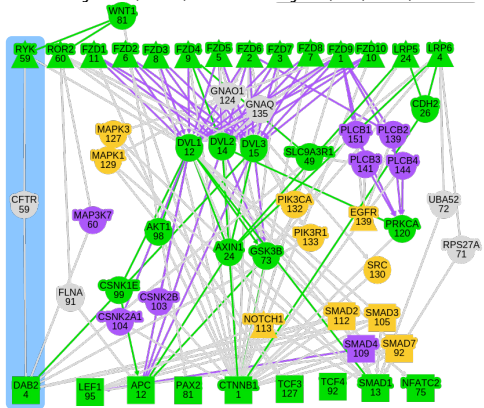


CS 5854: PATHLINKER

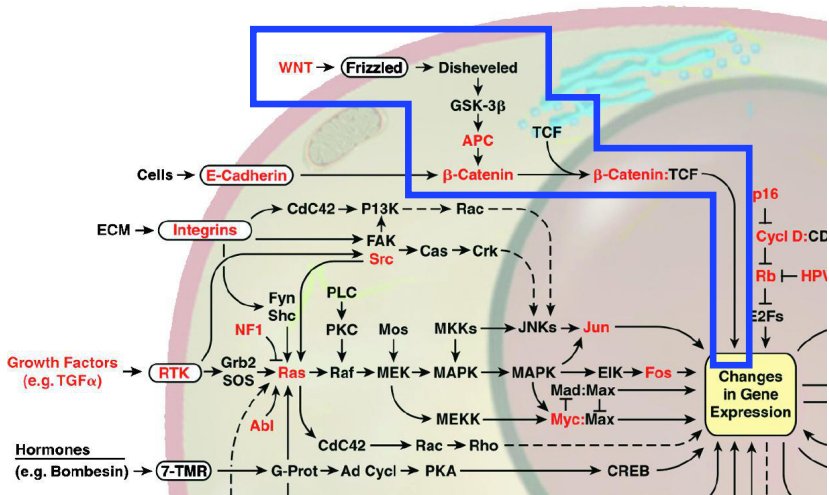
Automated Reconstruction of Human Signaling Networks

T. M. Murali

January 27, 29, February 3, 5, 10, 2025

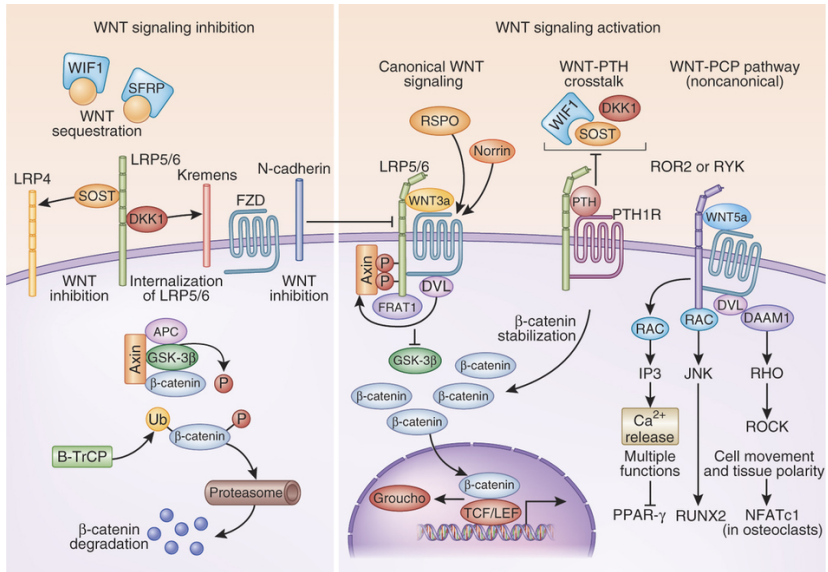


Wnt Pathway



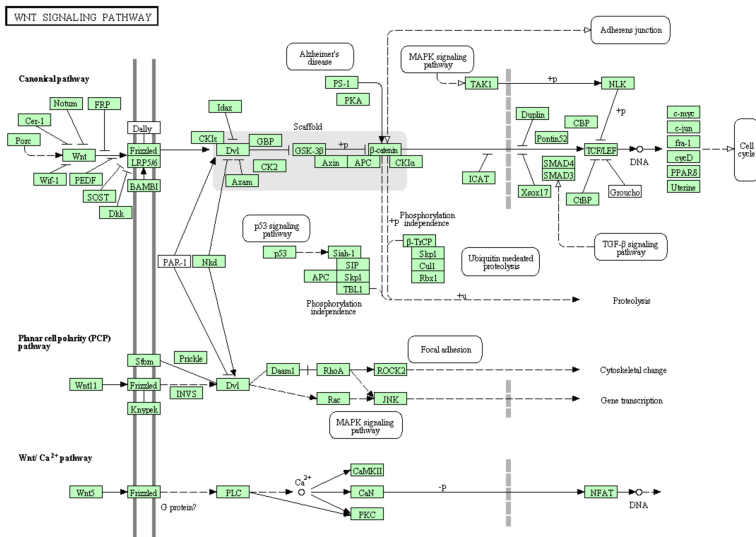
Hanahan and Weinberg. *Hallmarks of cancer*. Cell, 2000.

Wnt Pathway



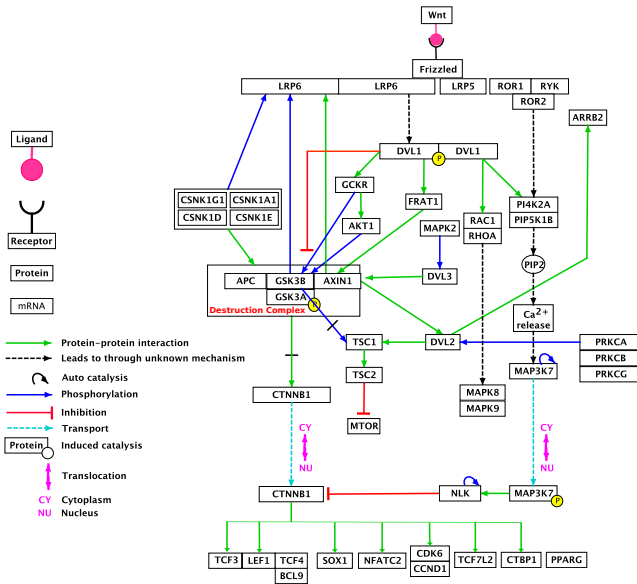
Baron and Kneissel. *WNT signaling in bone homeostasis and disease: from human mutations to treatments.* *Nat. Med.*, 2013.

Wnt Signaling in a Pathway Database

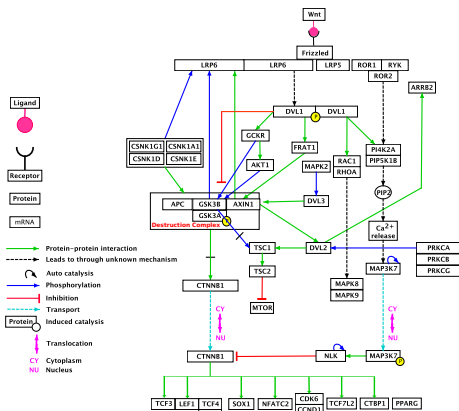


04310 34416
(c) Kanehisa Laboratories

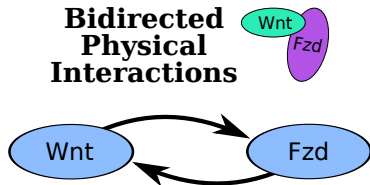
Wnt Signaling in a Pathway Database



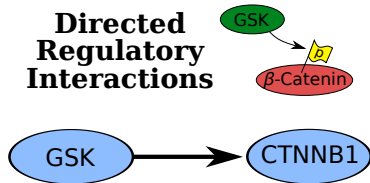
Signaling Pathways as Directed Graphs



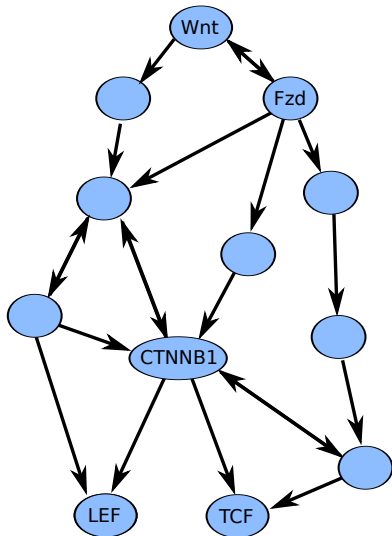
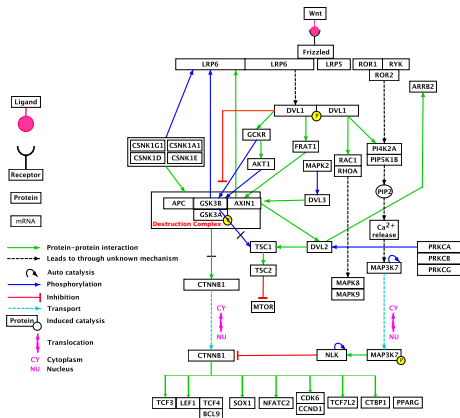
Bidirected Physical Interactions



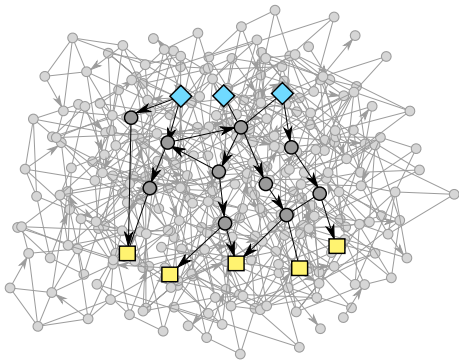
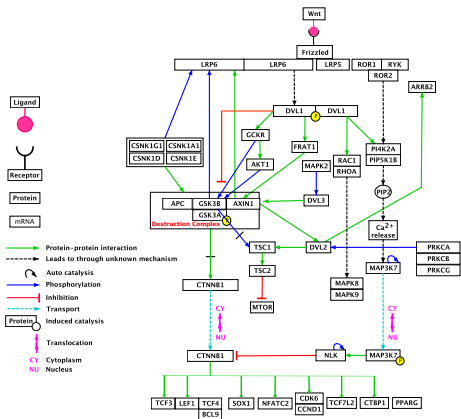
Directed Regulatory Interactions



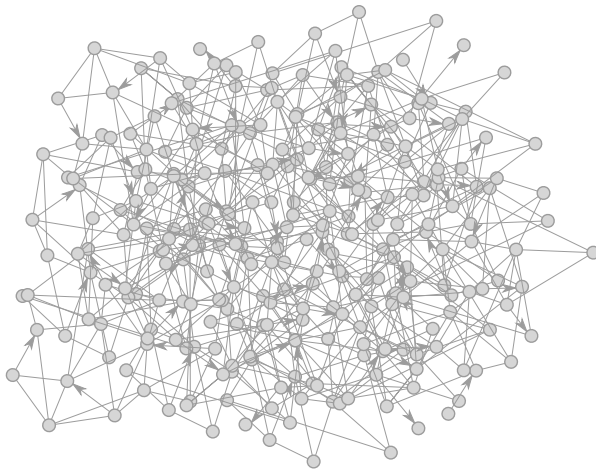
Signaling Pathways as Directed Graphs



Signaling Pathways as Directed Graphs

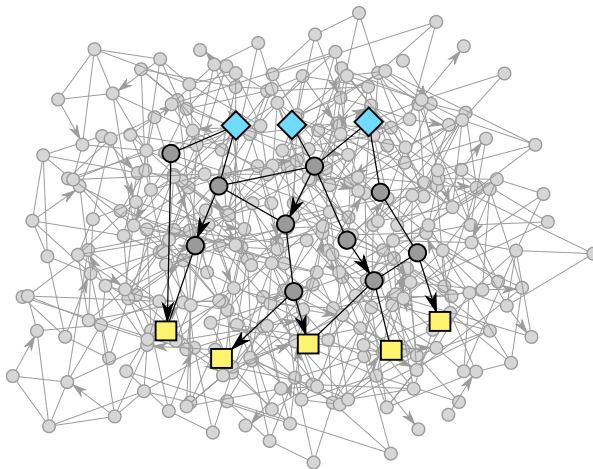


Reconstructing Signaling Pathways



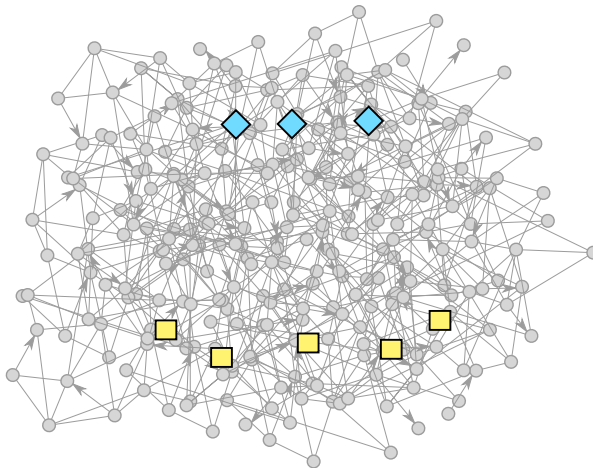
Human protein-protein interaction network
All known interactions among human proteins

Reconstructing Signaling Pathways



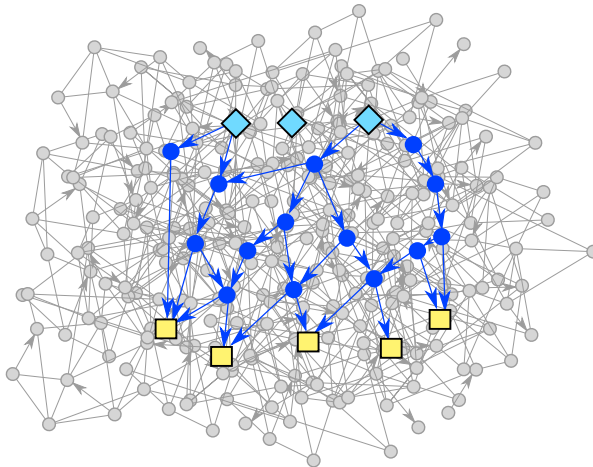
A pathway is a subgraph of the interaction network

Reconstructing Signaling Pathways



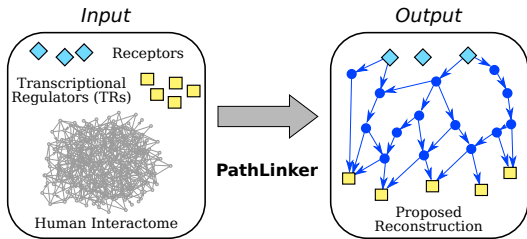
Question: Can we reconstruct the pathway given only receptors and transcriptional factors?

Reconstructing Signaling Pathways



Proposed pathway reconstruction

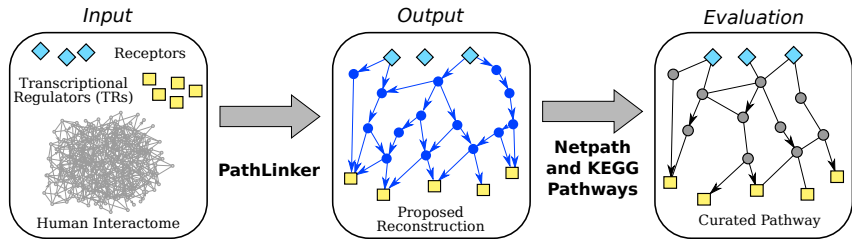
Automated Reconstruction of Signaling Pathways



- Developed **PathLinker** to reconstruct *proteins and interactions*
- **Systematically evaluated** PATHLINKER and other algorithms on human signaling pathways from the NetPath and KEGG databases

"Pathways on Demand: Automatic Reconstruction of Human Signaling Pathways," Ritz et al., *Systems Biology and Applications*, a Nature partner journal, 2, 16002, 2016.

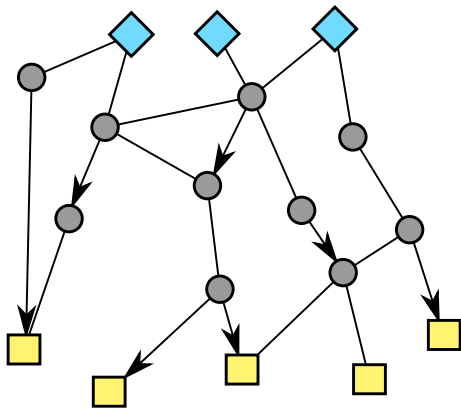
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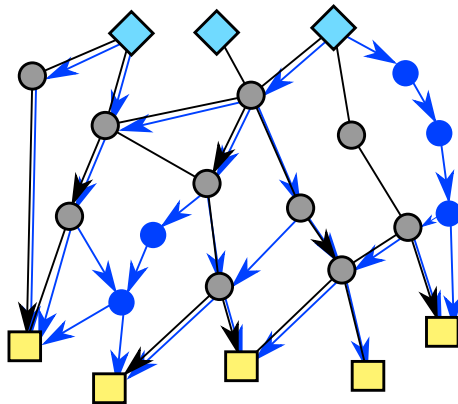
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Evaluation of Reconstructed Pathways



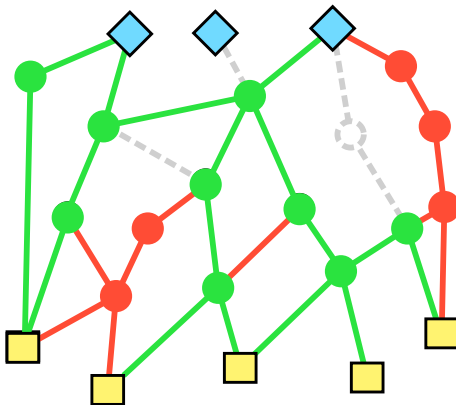
Curated Pathway

Evaluation of Reconstructed Pathways



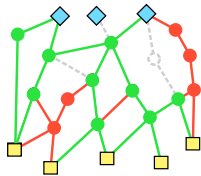
Curated Pathway and Proposed Reconstruction

Evaluation of Reconstructed Pathways



Curated Pathway and Proposed Reconstruction

Evaluation of Reconstructed Pathways

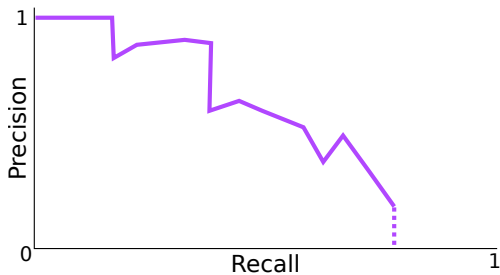


Recall:

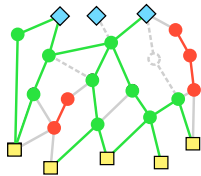
$$r_i = \frac{\text{true positives up to } i}{|P|}$$

Precision:

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Evaluation of Reconstructed Pathways

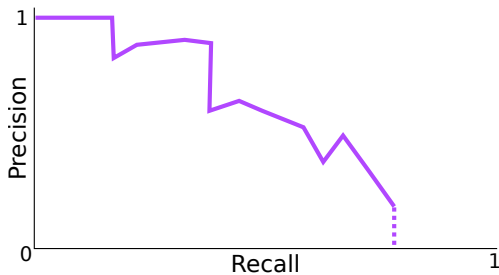


Recall:

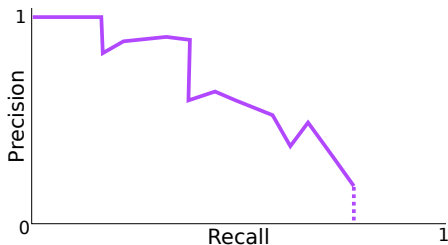
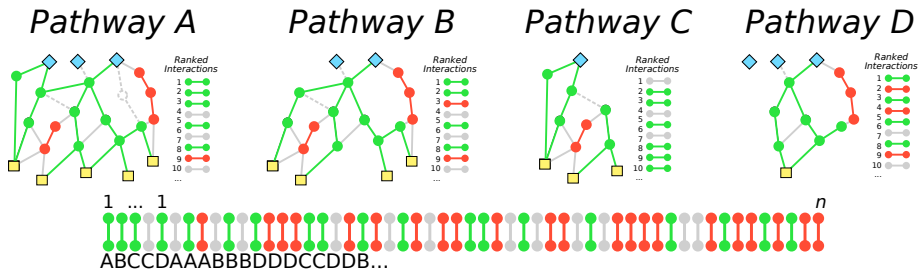
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Precision:

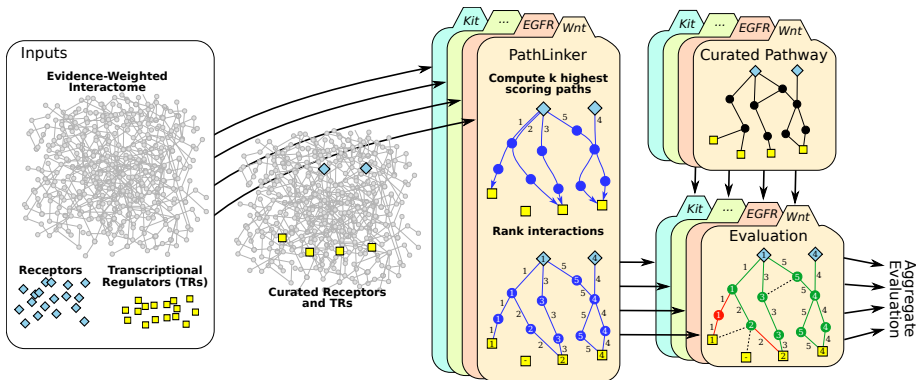
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Evaluating Multiple Reconstructions



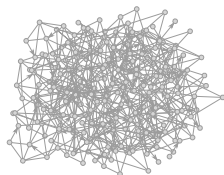
Complete Pipeline



Inputs for Pathway Reconstruction

Protein-Protein Interactome

- 12K nodes and 152K directed edges
- 61K **physical interactions**¹⁻⁴
BIND, DIP, InnateDB, IntAct, MINT, MatrixDB, Reactome, NetPath, KEGG, SPIKE
- 30K **signaling interactions**²⁻⁴
NetPath, KEGG, SPIKE



¹Aranda et al., *PSICQUIC and PSISSCORE: assessing and scoring molecular interactions*. *Nature Methods*, 2011.

²Kandasmy et al., *NetPath: a public resource of curated signaling transduction pathways*. *Genome Biology*, 2010.

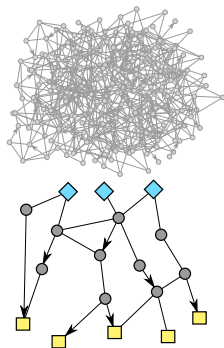
³Kanehisa et al., *KEGG for integration and interpretation of large-scale molecular data sets*. *Nucleic Acids Research*, 2012.

⁴Paz et al., *SPIKE: a database of highly curated human signaling pathways*. *Nucleic Acids Research*, 2009.

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NetPath, KEGG, SPIKE



Signaling Pathways from NetPath²

- 15 **immune and cancer** pathways

▶ [List of NetPath Pathways](#)

2,124 Receptors⁵

2,286 Transcriptional Regulators^{6,7}

¹Aranda et al., *PSICQUIC and PSISSCORE: assessing and scoring molecular interactions*. *Nature Methods*, 2011.

²Kandasmy et al., *NetPath: a public resource of curated signaling transduction pathways*. *Genome Biology*, 2010.

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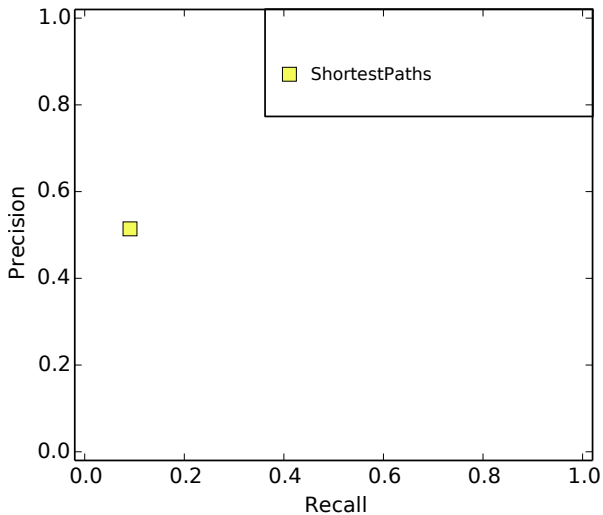
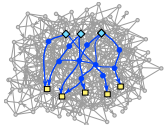
⁵Almen et al., *Mapping the human membrane proteome: a majority of the human membrane proteins can be classified according to function and evolutionary origin*. *BMC Biology*, 2009.

⁶Ravasi et al., *An atlas of combinatorial transcriptional regulation in mouse and man*. *Cell*, 2010.

⁷Vaquerizas et al., *A census of human transcription factors: function, expression and evolution*. *Nature Review Genetics*, 2009.

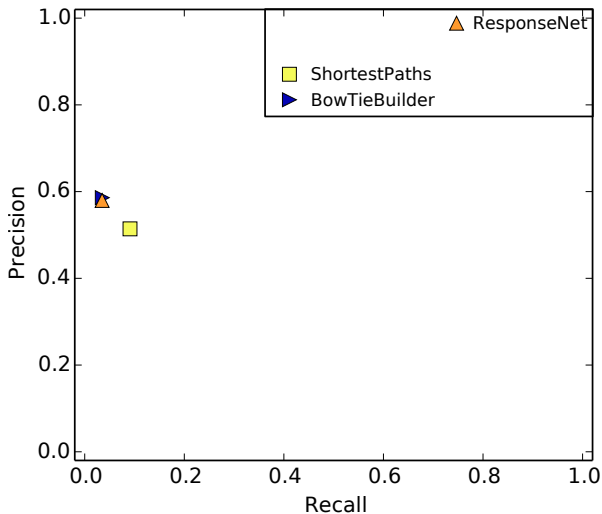
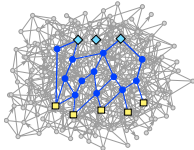
Results

SHORTESTPATHS



Results

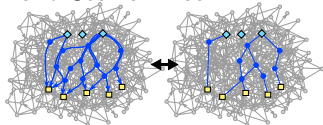
Network Flow:
RESPONSENET



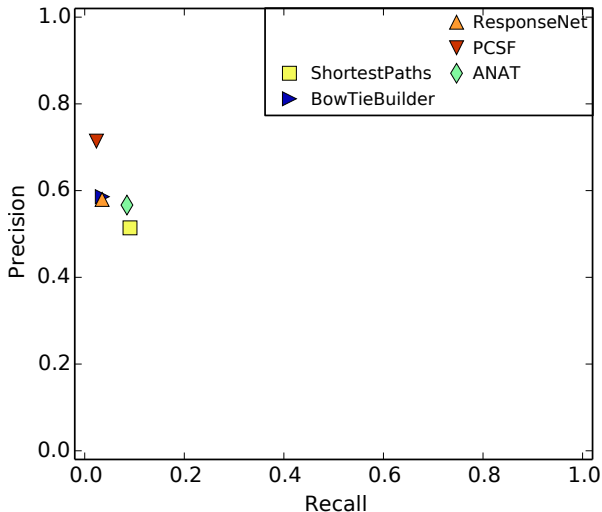
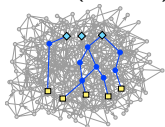
Yeger-Lotem et al., *Bridging high-throughput genetic and transcriptional data reveals cellular responses to alpha-synuclein toxicity*. *Nature Genetics*, 2009.

Results

Between SHORTESTPATHS
and Steiner Tree: ANAT



Prize Collecting Steiner
Forest (PCSF)

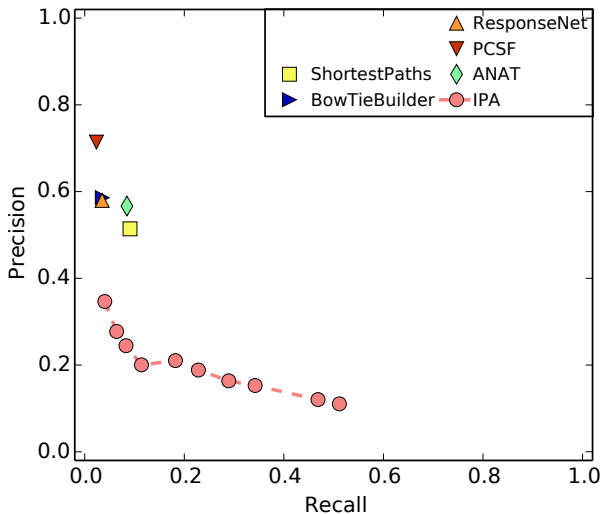
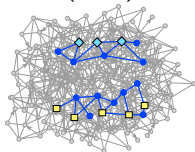


Yosef et al., ANAT: A tool for constructing and analyzing functional protein networks. *Science Signaling*, 2011.

Tuncbag et al., Simultaneous reconstruction of multiple signaling pathways via the prize-collecting Steiner forest problem. *Journal of Computational Biology*, 2013.

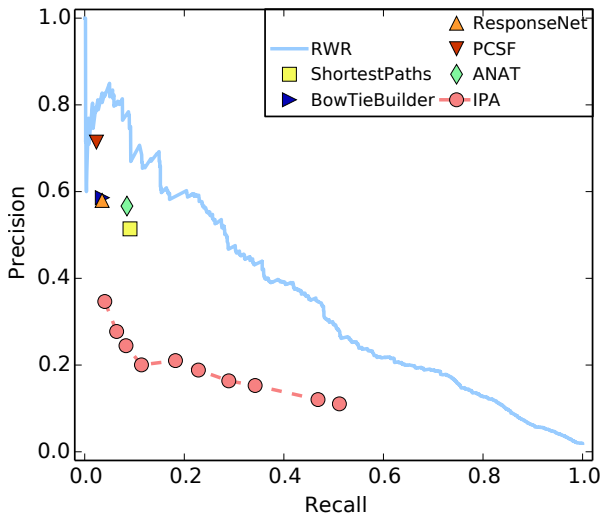
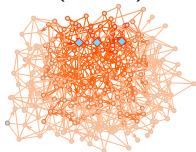
Results

Ingenuity Pathway Analyzer (IPA)



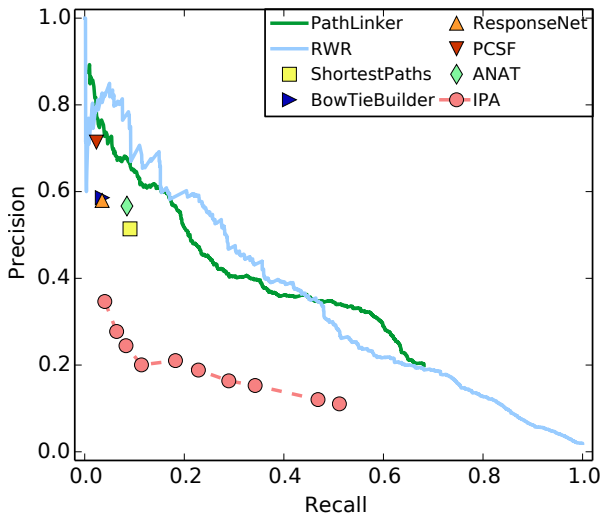
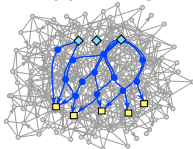
Results

Random Walk with Restarts
(RWR)



Results

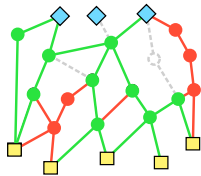
PathLinker



Yen. Finding the k shortest loopless paths in a network. *Management Science*, 1971.

This paper.

Evaluation of Reconstructed Pathways

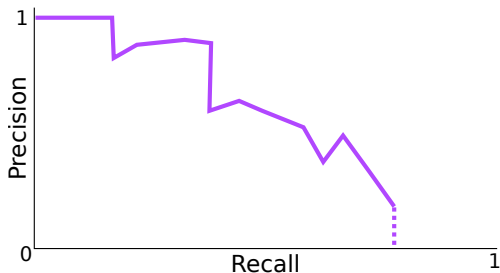


Recall:

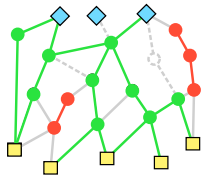
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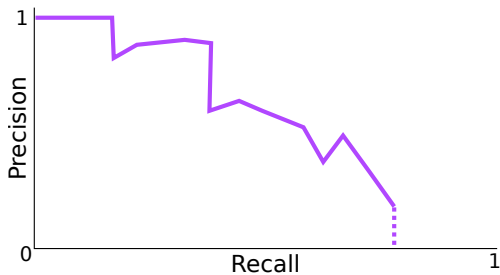


Recall:

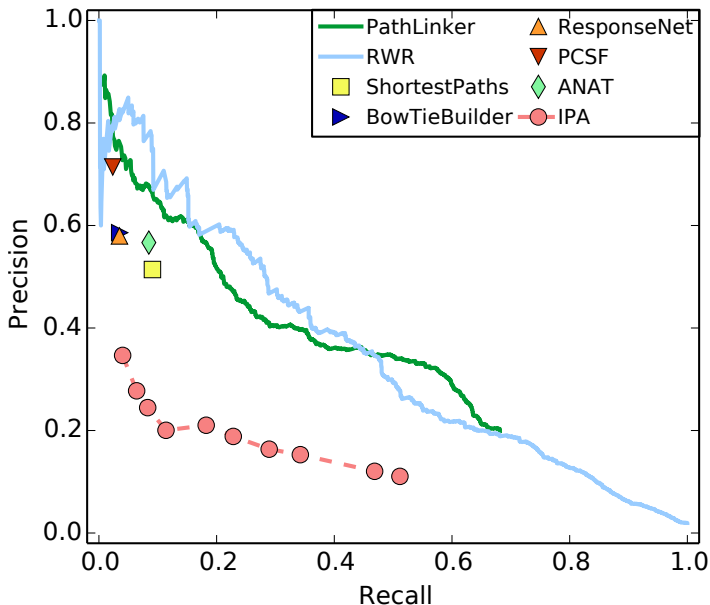
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Precision:

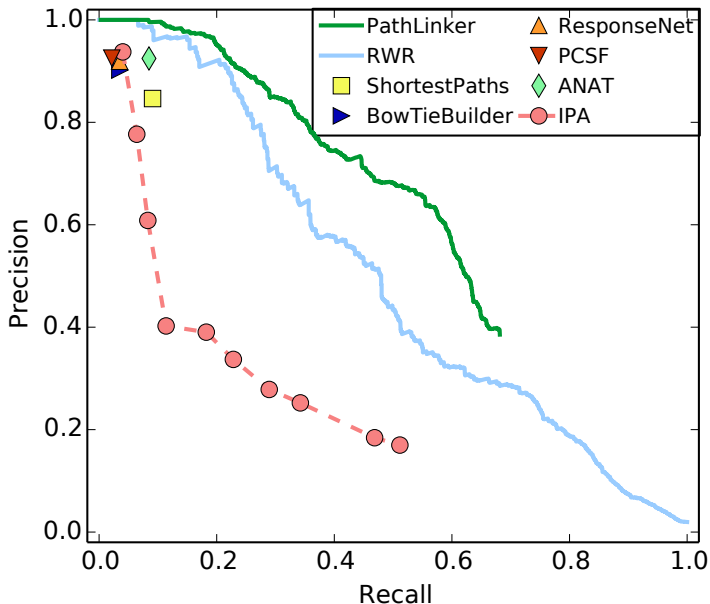
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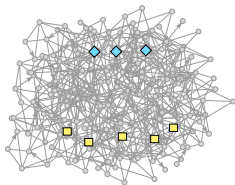
Ignore Pathway-Adjacent Negatives



Ignore Pathway-Adjacent Negatives

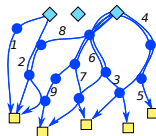


Why does PathLinker improve over other methods?

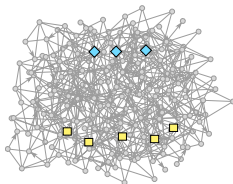


PathLinker

Compute k
highest
scoring paths

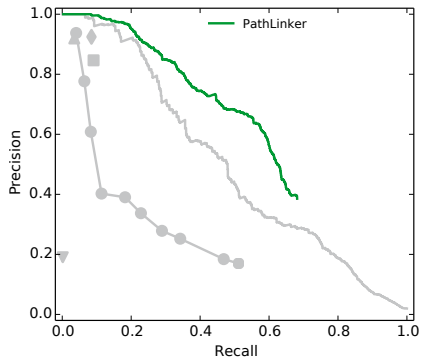
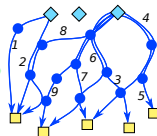


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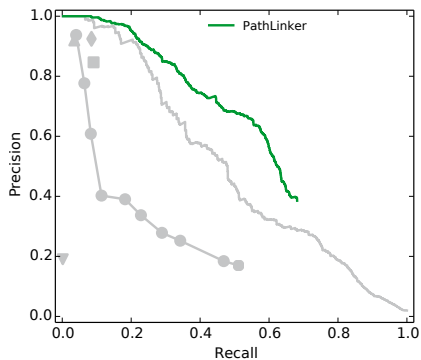
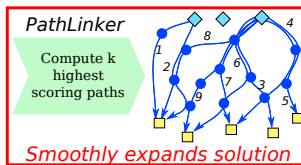
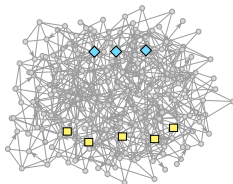


PathLinker

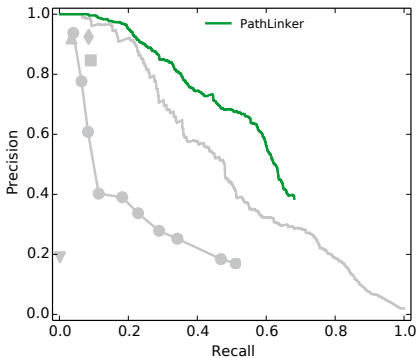
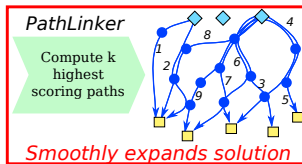
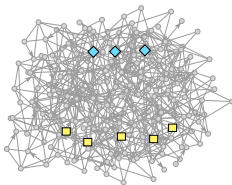
Compute k
highest
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Why does PathLinker improve over other methods?



Why does PathLinker improve over other methods?



► Parameter sweep

Algorithms Compared

Abbreviation	Algorithm Type
SHORTESTPATHS	Shortest paths from every receptor to every TR
PATHLINKER	k shortest paths from any receptor to any TR
RWR	Random walk with restarts (aka PageRank)
RESPONSENET	Network flow
ANAT	Tradeoff between shortest paths and Steiner trees
PCSF	Prize-Collecting Steiner Forest
IPA	Ingenuity Pathway Analyzer: grow subnetworks greedily
BOWTIEBUILDER	Approximation to the Steiner tree connecting receptors and TRs

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Drunkard's Walk

- A drunk person leaves a bar.
- They move in steps, either by one unit to the right or by one unit to the left.
- When will they reach their home at the end of the street?
- If they return to the bar, they can only step out.

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- They move in steps, either by one unit to the right or by one unit to the left.
- When will they reach their home at the end of the street?
- If they return to the bar, they can only step out.
- How do we think about this problem?
- Street is the x -axis, bar is at $x = 0$, house is at $x = n$.
- Where could the drunk be after 1 step? After 2 steps? After 3 steps? After k steps?
- What is the probability that the drunk reaches home after k steps?
- What is the probability that the drunk reaches home at all?

Random Walk on a Grid

- A random walker leaves a starting location (conveniently at $(0,0)$).
- They move in steps, either by one unit to the right, left, top, or bottom.
- When will they reach their destination, which is at (n, n) ?

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Random Walk on a Grid

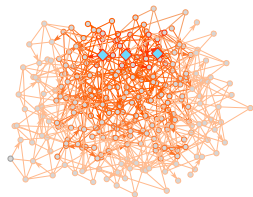
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- Where could the walker be after 1 step? After 2 steps? After 3 steps? After k steps?
- What is the probability that the walker reaches their destination after k steps?
- Convenient to think of the grid as a graph. **Can generalise the problem to a graph.**

RWR Algorithm

Given weighted, directed graph $G = (V, E, W)$, receptors $S \subset V$ and TRs $T \subset V$, and a parameter $0 \leq q < 1$.

- Walker at u transitions as follows:

Walk: With prob. $1 - q$, walk to neighbor x with prob. w_{ux}/d_u (outdegree)



Page et al., The PageRank citation ranking: Bringing order to the web. Technical Report, 1999.

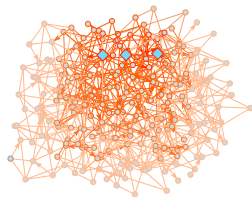
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Teleport: With prob. q , teleport to one of $s \in S$, selected uniformly at random



Page et al., The PageRank citation ranking: Bringing order to the web. Technical Report, 1999.

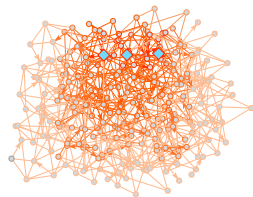
RWR Algorithm

Given weighted, directed graph $G = (V, E, W)$, receptors $S \subset V$ and TRs $T \subset V$, and a parameter $0 \leq q < 1$.

- Walker at u transitions as follows:

Walk: With prob. $1 - q$, walk to neighbor x with prob. w_{ux}/d_u (outdegree)

Teleport: With prob. q , teleport to one of $s \in S$, selected uniformly at random



- Compute the probability $p(v)$ each node is visited as steps $\rightarrow \infty$.

$$p(v) = \frac{q}{|S|} [v \in S] + (1 - q) \sum_{u \in N_v^{\text{in}}} \frac{w_{uv}}{d_u} p(u).$$

Page et al., *The PageRank citation ranking: Bringing order to the web. Technical Report, 1999.*

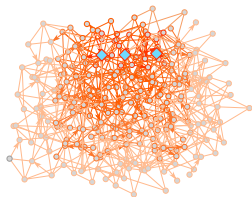
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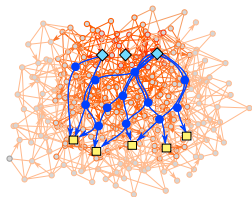
- Output edges in decreasing order of **edge fluxes**: $f_{uv} = p_u w_{uv}$

Page et al., The PageRank citation ranking: Bringing order to the web. Technical Report, 1999.

PathLinker Algorithm

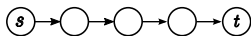
Given weighted, directed graph $G = (V, E, W)$, receptors $S \subset V$ and TRs $T \subset V$.

- Find the k “highest-scoring” paths from any $s \in S$ to any $t \in T$.
- Replace Dijkstra’s algorithm with the A* algorithm for significant practical speedup.



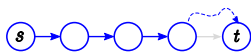
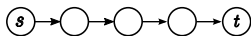
Yen. *Finding the k shortest loopless paths in a network*. *Management Science*, 1971.

Shortest Loopless Paths – Basic Idea



- Naïve Approaches (time-consuming):
 - ▶ Enumerate all paths from s to t and sort.
 - ▶ Obtain $k - 1$ shortest paths, hide an edge from each path and find a shortest path in the modified network. Test all combinations.

Shortest Loopless Paths – Basic Idea



- Naïve Approaches (time-consuming):
 - ▶ Enumerate all paths from s to t and sort.
 - ▶ Obtain $k - 1$ shortest paths, hide an edge from each path and find a shortest path in the modified network. Test all combinations.
- Basic idea of Yen's algorithm:
 - ▶ Compute the shortest path from s to t
 - ▶ The k^{th} shortest path will be a deviation from the previously-discovered shortest path.

Shortest Loopless Paths

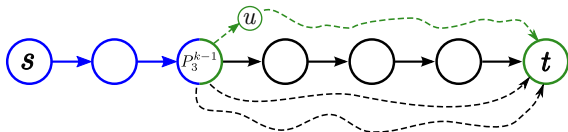
- $\{s, v_2, v_3, \dots, t\}$ denotes a simple path from s to t
- $P^k = \{s, P_2^k, P_3^k, \dots, P_{|P^k|-1}^k, t\}$ is the k^{th} shortest path from s to t

Shortest Loopless Paths

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- $P^k = \{s, P_2^k, P_3^k, \dots, P_{|P^k|-1}^k, t\}$ is the k^{th} shortest path from s to t
- D_i^k is the “deviation from P^{k-1} at node P_i^{k-1} ”

More specifically, the shortest $s \rightsquigarrow t$ path that:

- 1 coincides with P^{k-1} from s to P_i^{k-1}
- 2 deviates to a node u where u is not used as this deviation in any of the $k-1$ shortest paths
- 3 reaches t by a shortest path from u without using any node in the first part of the path

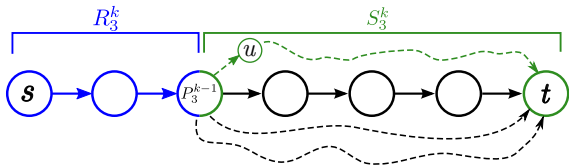


Shortest Loopless Paths

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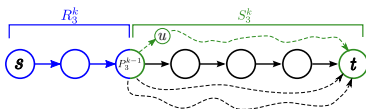
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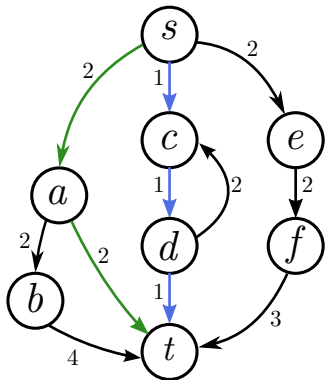
- $R_i^k = \{s, P_2^k, P_3^k, \dots, P_i^k\}$ is the root of D_i^k
- $S_i^k = \{P_i^k, \dots, t\}$ is the spur of D_i^k

Shortest Loopless Paths

- Find the shortest path P^1
- For $k = 2, 3, \dots$, find P^k as follows:
 - 1: Let $B^k = B^{k-1}$, the set of candidate paths from iteration $k - 1$
 - 2: **for** $1 \leq i < |P^{k-1}|$ **do**
 - 3: Let $x = P_i^{k-1}$
 - 4: Hide incoming edges to x for the remainder of iteration k
 - 5: **for** each j such that the first i nodes in P^j match P^{k-1} **do**
 - 6: Hide edge (x, P_{i+1}^j) for the remainder of iteration k
 - 7: **end for**
 - 8: R_i^k is the first i nodes of P^{k-1}
 - 9: S_i^k is the shortest path from x to t
 - 10: Join R_i^k and S_i^k to form D_i^k
 - 11: Add candidate path D_i^k to B^k
 - 12: **end for**
 - 13: Remove the shortest path from B^k and return it



Example – Find P^3

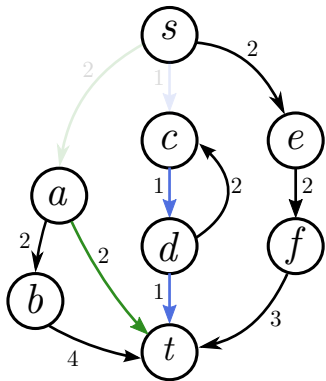


$$P^1 = \{s, c, d, t\}$$

$$P^2 = \{s, a, t\}$$

$$P^3 = ?$$

Example – Hide Edges for Root $\{s\}$

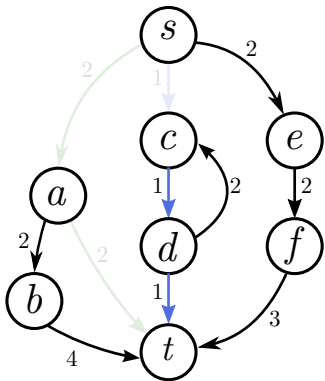


$$P^1 = \{s, c, d, t\}$$

$$P^2 = \{s, a, t\}$$

$$P^3 = ?$$

Example – Hide Edges for Root $\{s, a\}$

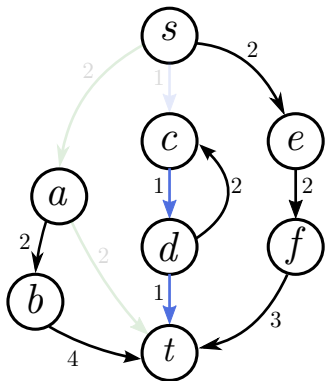


$$P^1 = \{s, c, d, t\}$$

$$P^2 = \{s, a, t\}$$

$$P^3 = ?$$

Example – Find Shortest Spur for Each Root



$$P^1 = \{s, c, d, t\}$$

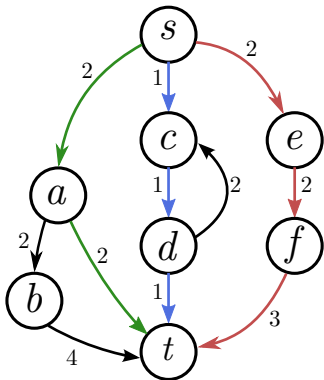
$$P^2 = \{s, a, t\}$$

$$P^3 = ?$$

$$S_1^3 = \{s, e, f, t\}$$

$$S_2^3 = \{a, b, t\}$$

Example – Identify Shortest Deviation



$$P^1 = \{s, c, d, t\}$$

$$P^2 = \{s, a, t\}$$

$$P^3 = ?$$

$$S_1^3 = \{s, e, f, t\}$$

$$S_2^3 = \{a, b, t\}$$

$$D_1^3 = \{s, e, f, t\}$$

$$D_2^3 = \{s, a, b, t\}$$

How do we find S_i^k efficiently?

- For $k = 2, 3, \dots$, find P^k as follows:
 - 1: Let $B^k = B^{k-1}$, the set of candidate paths from iteration $k - 1$
 - 2: **for** $1 \leq i < |P^{k-1}|$ **do**
 - 3: Let $x = P_i^{k-1}$
 - 4: Hide incoming edges to x for the remainder of iteration k
 - 5: **for** each j such that the first i nodes in P^j match P^{k-1} **do**
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 - 7: **end for**
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 - 9: S_i^k is the shortest path from x to t
 - 10: Join R_i^k and S_i^k to form D_i^k
 - 11: Add candidate path D_i^k to B^k
 - 12: **end for**
 - 13: Remove the shortest path from B^k and return it

Compare Distances to Curated Pathway

Distance of interactions from
any node in the pathway

Recall = 0.20



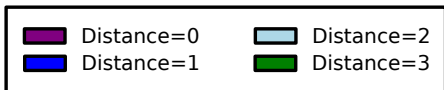
Recall = 0.40



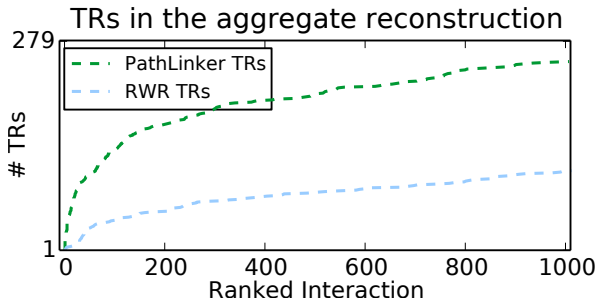
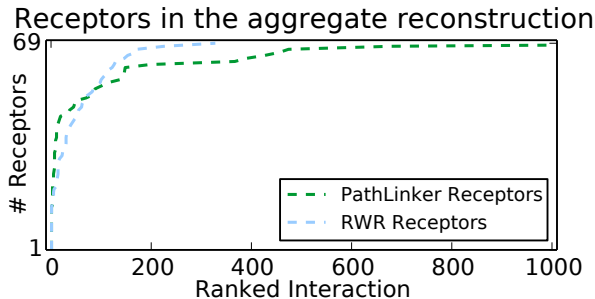
Recall = 0.60



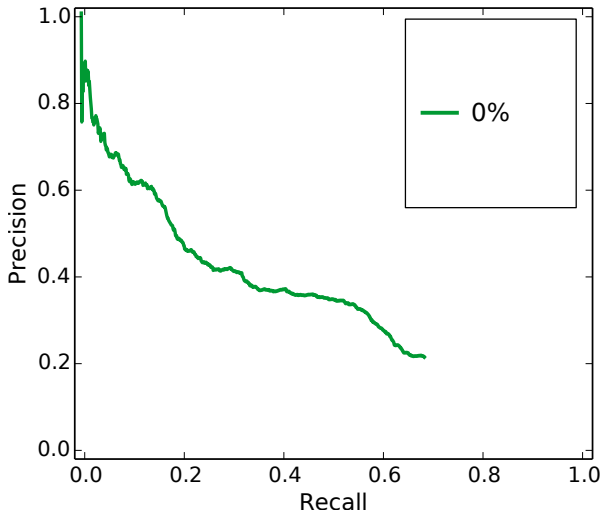
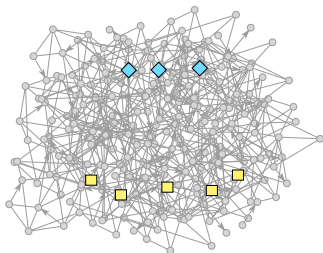
Proportion of Interactions



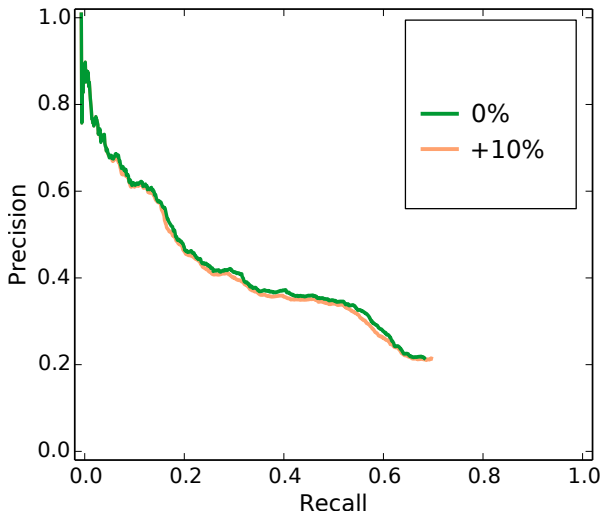
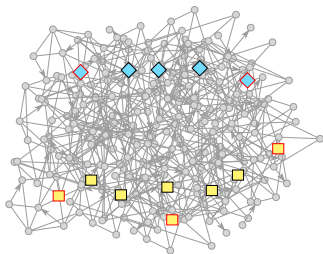
Compare Rate of Recovery of Receptors/TRs



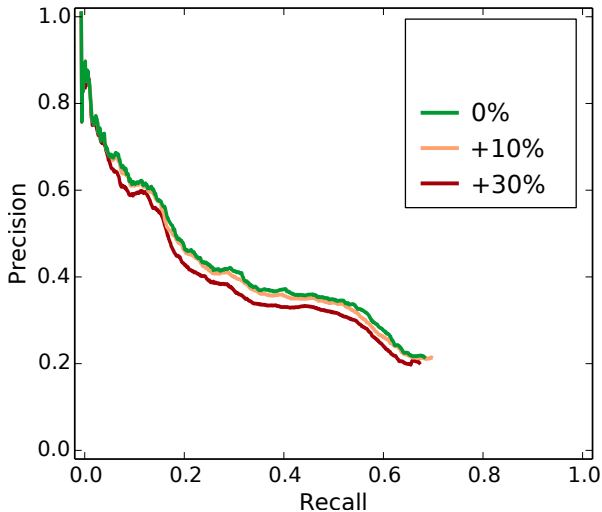
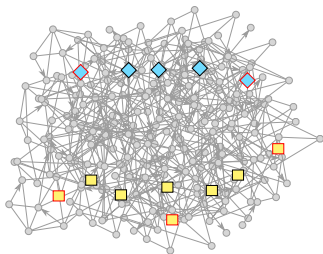
Add Noise to Inputs



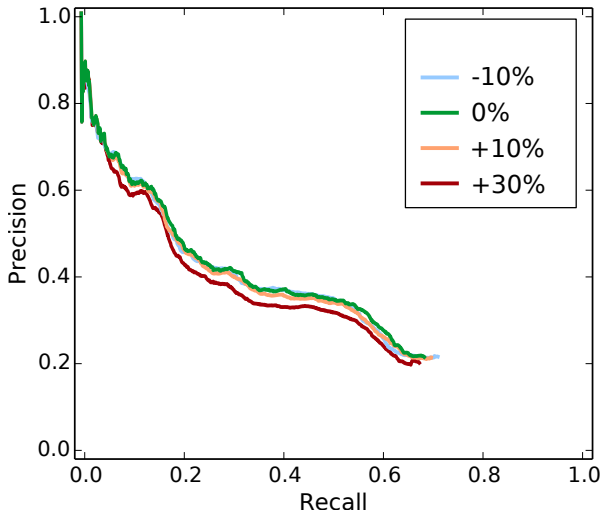
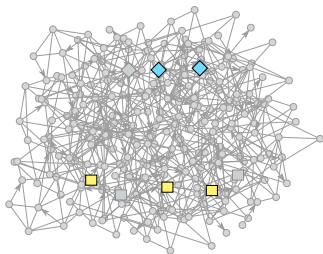
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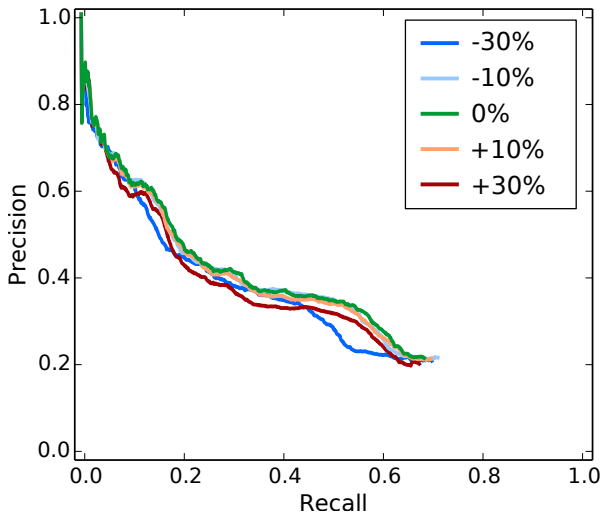
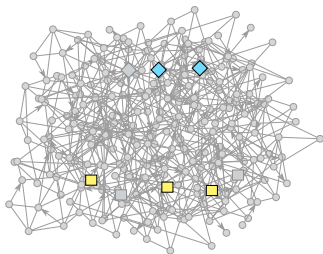
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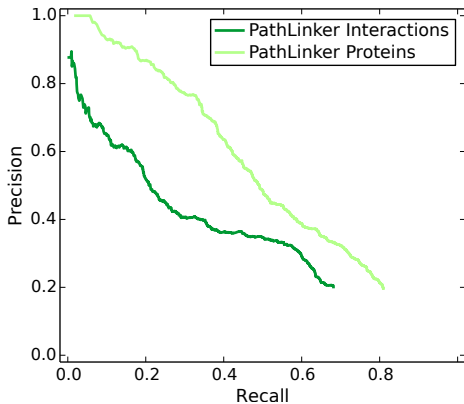
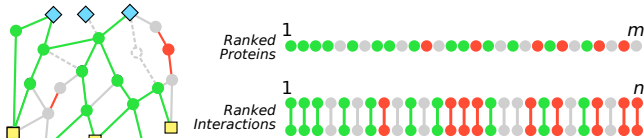
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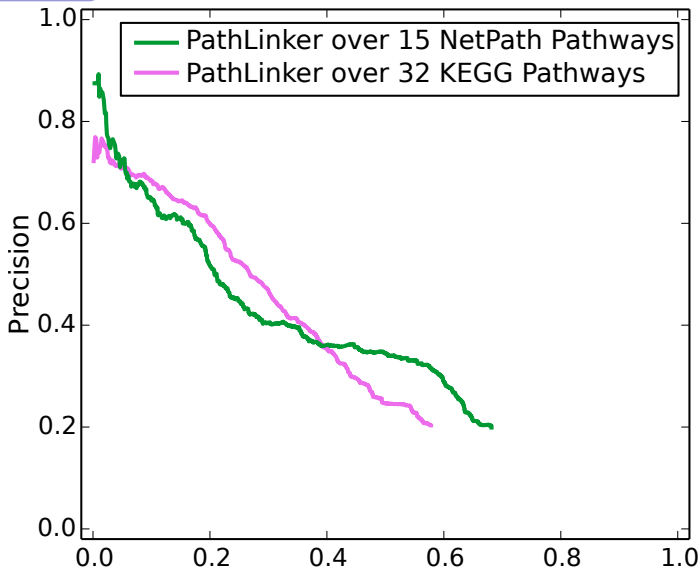


Compare to Reconstructing Proteins



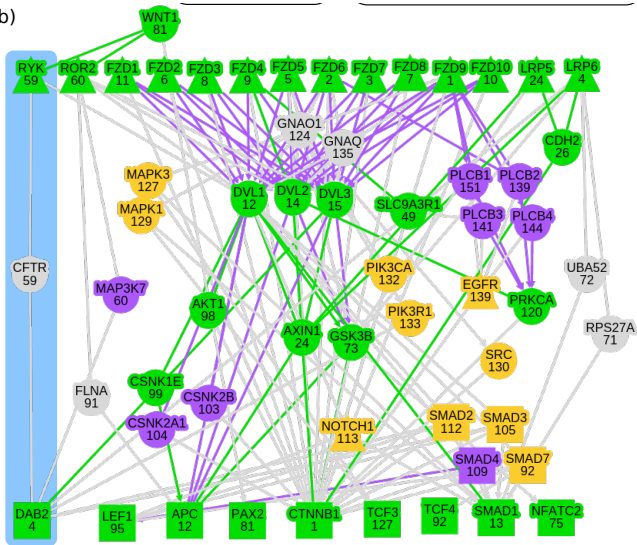
Reconstruct KEGG Pathways

► List of KEGG Pathways

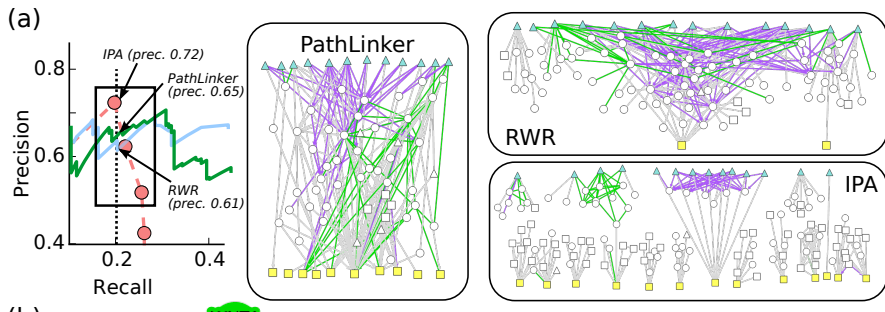


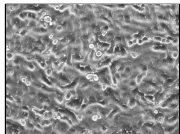
Wnt Signaling Pathway: Top 200 PathLinker Paths

(b)



Comparing Wnt Reconstructions





HEK293 Cells

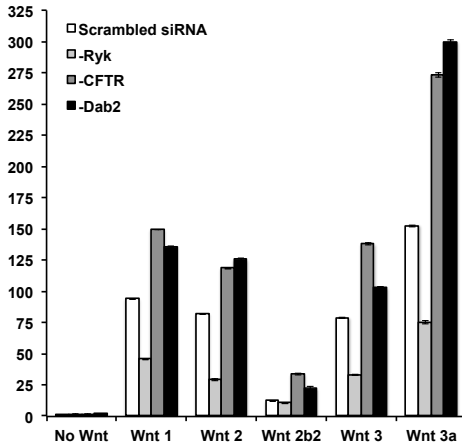
Day 0

Plate cells and
siRNA silence
gene of interest

Day 1

Transfect secreted
Wnt3a (sWnt3a) stimulus
and Luciferase reporter

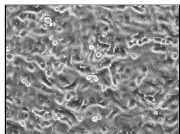
Day 3

Measure
luminescenceNormalized Relative
Luminescence

▶ Luciferase Reporter Efficacy

▶ siRNA Silencing Efficacy

▶ ColP



HEK293 Cells

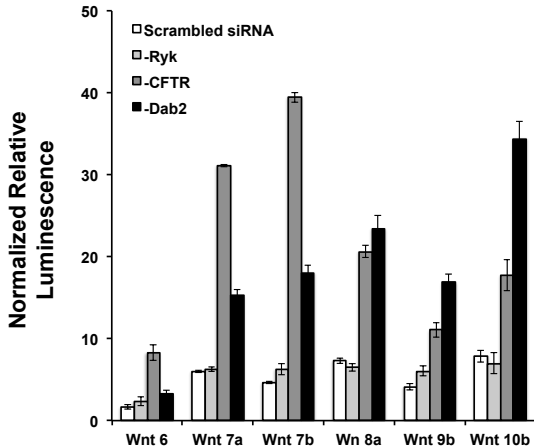
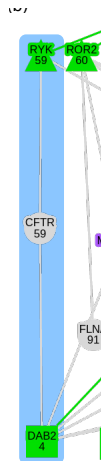
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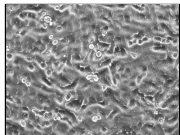
Day 3

Measure
luminescence

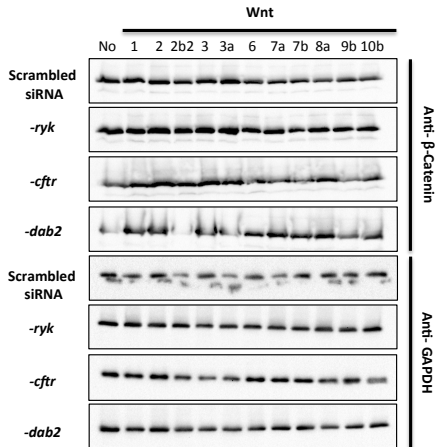
▶ Luciferase Reporter Efficacy

▶ siRNA Silencing Efficacy

▶ ColP



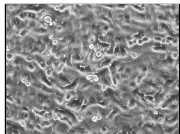
HEK293 Cells

Day
0Plate cells and
siRNA silence
gene of interestDay
1Transfect secreted
Wnt3a (sWnt3a) stimulusDay
3Measure
cellular **B-catenin**
levels

▶ Luciferase Reporter Efficacy

▶ siRNA Silencing Efficacy

▶ ColP



HEK293 Cells

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Plate cells and
siRNA silence
gene of interest

Day 1

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Wnt3a (sWnt3a) stimulus

Day 3

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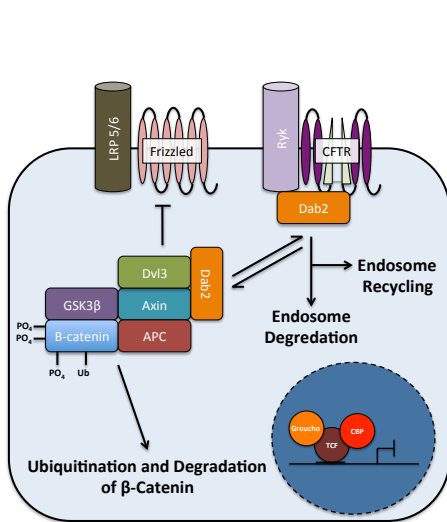
Wnt	Control		-Ryk		-CFTR		-Dab2	
	NRL	QNβ	NRL	QNβ	NRL	QNβ	NRL	QNβ
No Wnt	--	--	--	--	--	--	--	--
Wnt 1	VS	++	S	-	VS	+	VS	++
Wnt 2	VS	+	S	++	VS	++	VS	++
Wnt 2b2	W	-	W	+	S	++	W	-
Wnt 3	VS	++	S	++	VS	++	VS	++
Wnt 3a	VS	++	VS	++	VS	++	VS	++
Wnt 6	W	++	W	+	W	+	W	++
Wnt 7a	W	-	W	+	S	-	W	++
Wnt 7b	W	++	W	-	S	-	W	++
Wnt 8a	W	-	W	-	W	++	W	++
Wnt 9b	W	-	W	-	W	-	W	++
Wnt 10b	W	-	W	-	W	++	S	++

▸ Luciferase Reporter Efficacy

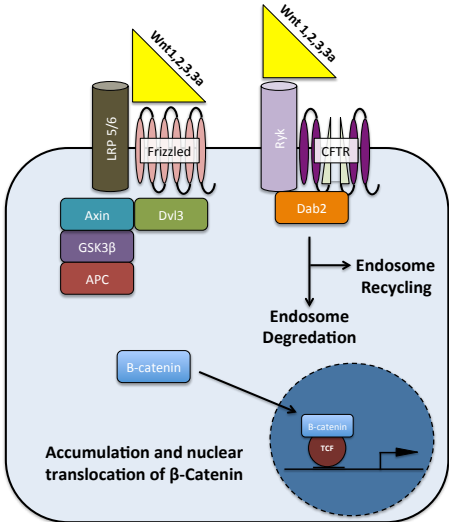
▸ siRNA Silencing Efficacy

▸ ColP

New Model: Dvl is an Amplifier of Wnt Signaling

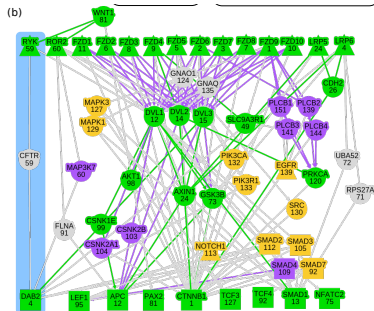
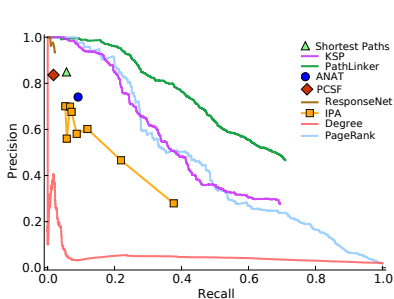
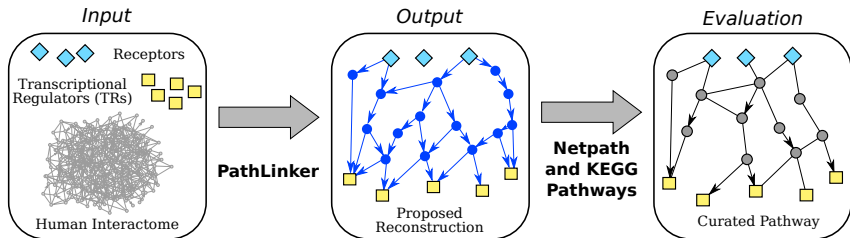


- Wnt Signaling

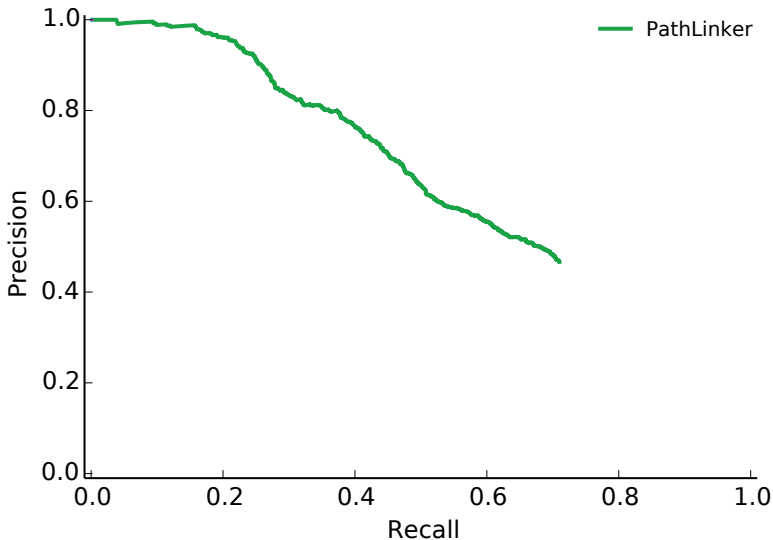


+ Wnt 1,2,3,3a Signaling

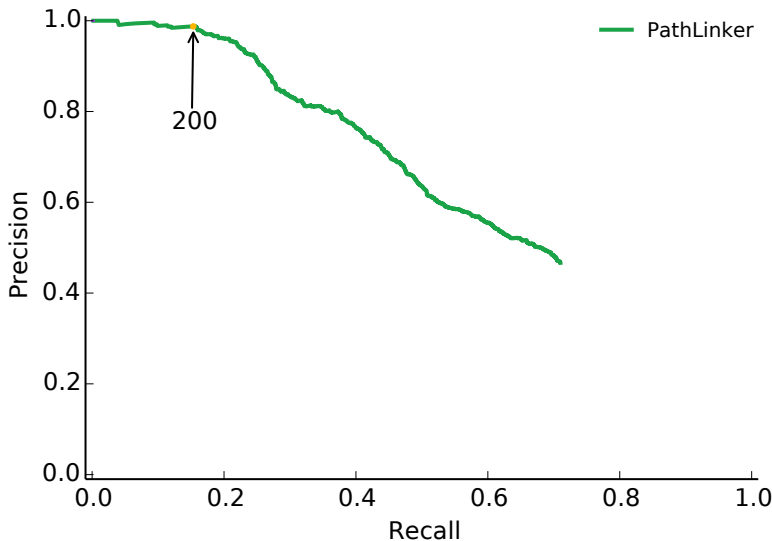
PathLinker Summary



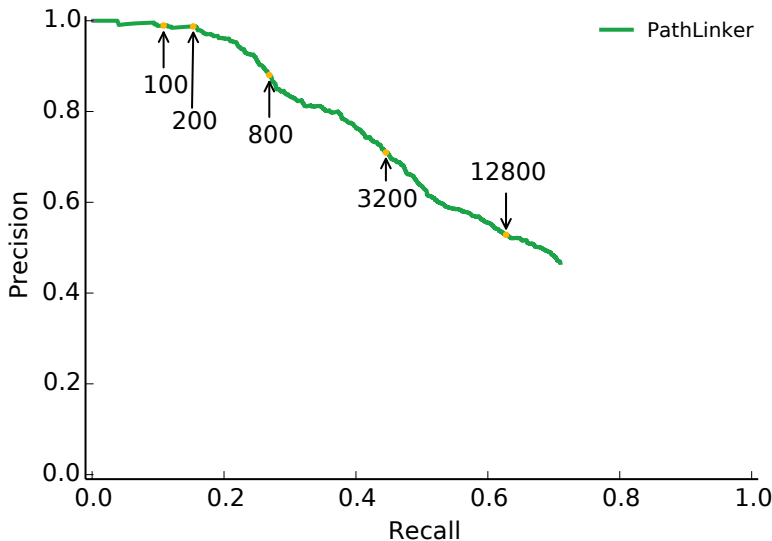
How Many Paths Does PathLinker Need to Compute?



How Many Paths Does PathLinker Need to Compute?



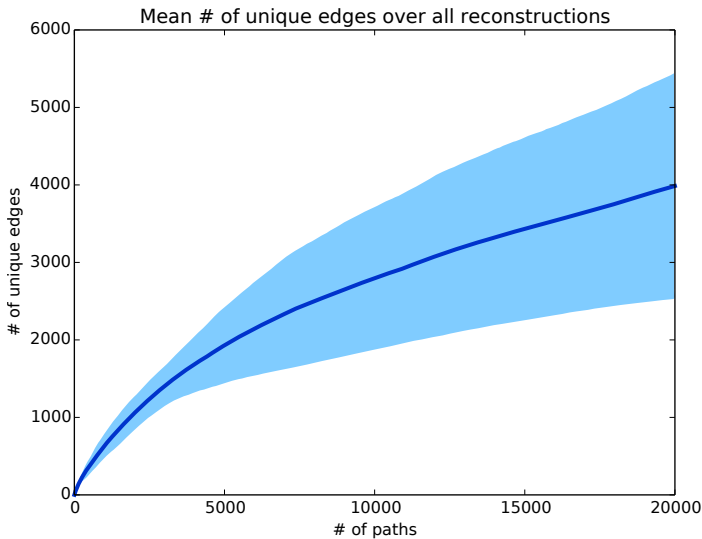
How Many Paths Does PathLinker Need to Compute?



We compute **20,000** paths to achieve a recall of 0.7.

► Performance

How Many Paths Does PathLinker Need to Compute?



We compute **20,000** paths to achieve a recall of 0.7.

► Performance

NetPath Pathways

- 1 The pathway contains at least one receptor.
- 2 The pathway contains at least one TR, and
- 3 The minimum cut between the receptors and TRs was at least three in the NetPath pathway.

Pathway	#Nodes	#Edges	Min Cut	# Receptors	# TRs
BDNF	72	139	4	5	4
EGFR1	231	1456	30	6	33
IL1	43	178	7	3	5
IL2	67	242	16	3	12
IL3	70	176	5	2	9
IL6	53	162	6	4	14
IL7	18	52	5	2	3
Kit Receptor	76	207	5	6	8
Leptin	55	135	8	3	15
Prolactin	68	199	10	4	9
RANKL	57	142	4	2	12
TCR	154	504	8	4	21
TGF β Receptor	209	863	32	5	78
TNF α	239	913	15	4	44
Wnt	106	428	7	14	14

◀ Inputs for Pathway Reconstruction

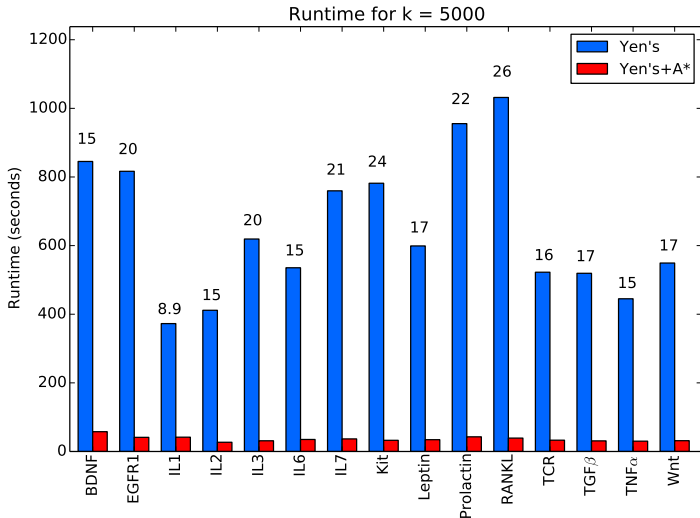
KEGG Pathways

[← KEGG Results](#)

- 1 The Pathway is related to signaling.
- 2 The pathway contains at least one receptor.
- 3 The pathway contains at least one TR, and
- 4 The minimum cut between the receptors and TRs was ≥ 3 in the KEGG pathway.

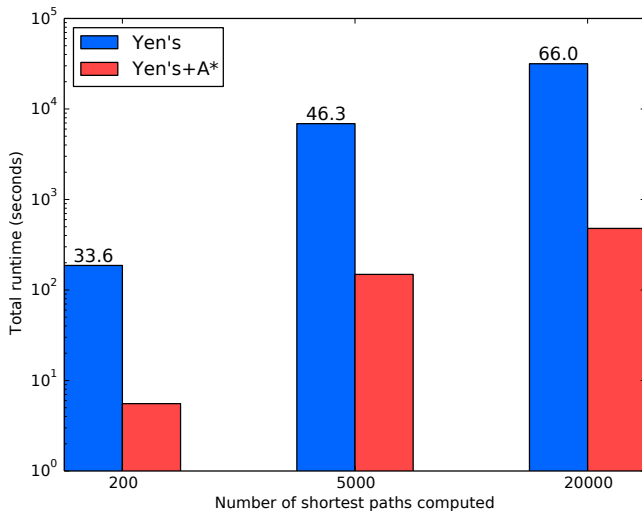
Name	KEGG ID	Name	KEGG ID
Adherens junction	hsa04520	Adipocytokine signaling pathway	hsa04920
Apoptosis	hsa04210	Axon guidance	hsa04360
Chemokine signaling pathway	hsa04062	Circadian entrainment	hsa04713
Dopaminergic synapse	hsa04728	Endocytosis	hsa04144
ErbB signaling pathway	hsa04012	Focal adhesion	hsa04510
FoxO signaling pathway	hsa04068	GnRH signaling pathway	hsa04912
HIF-1 signaling pathway	hsa04066	Hippo signaling pathway	hsa04390
Insulin signaling pathway	hsa04910	Jak-STAT signaling pathway	hsa04630
Prolactin signaling pathway	hsa04917	MAPK signaling pathway	hsa04010
Melanogenesis	hsa04916	Natural killer cell mediated cytotoxicity	hsa04650
Neurotrophin signaling pathway	hsa04722	NF-kappa B signaling pathway	hsa04064
Notch signaling pathway	hsa04330	Osteoclast differentiation	hsa04380
TGF-beta signaling pathway	hsa04350	Thyroid hormone signaling pathway	hsa04919
Tight junction	hsa04530	Toll-like receptor signaling pathway	hsa04620
VEGF signaling pathway	hsa04370	Wnt signaling pathway	hsa04310
Leukocyte transendothelial migration	hsa04670	Signaling pathways regulating pluripotency of stem cells	hsa04550

PathLinker Performance



► How Many Paths?

PathLinker Performance



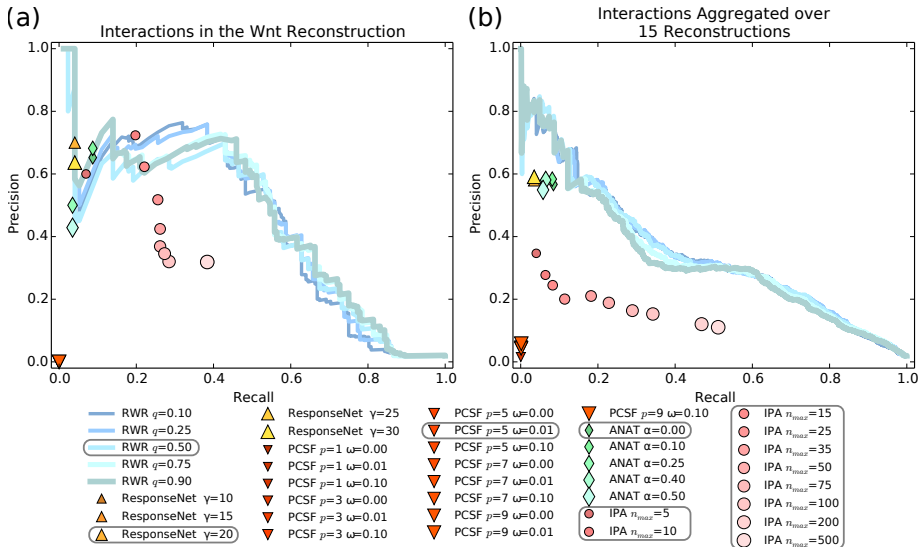
► How Many Paths?

Algorithm Internal Parameters

Algorithm	Parameter	Meaning
PathLinker	k	Number of shortest paths
RWR	q	Teleportation probability
ANAT	α	Tradeoff between global (Steiner tree) and local (shortest path) solution
PCSF	ω	Penalty for adding a new tree
	p	Prize for each node
ResponseNet	γ	Number of interactions that carry flow
IPA	n_{max}	Maximum sub-network size

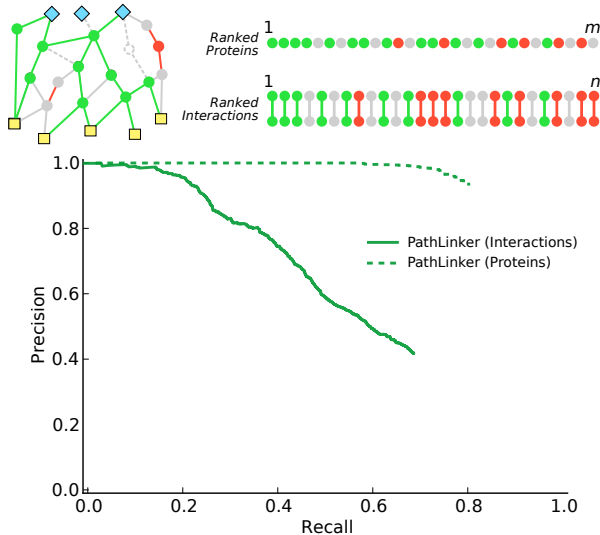
◀ PathLinker Summary

Algorithm Internal Parameters

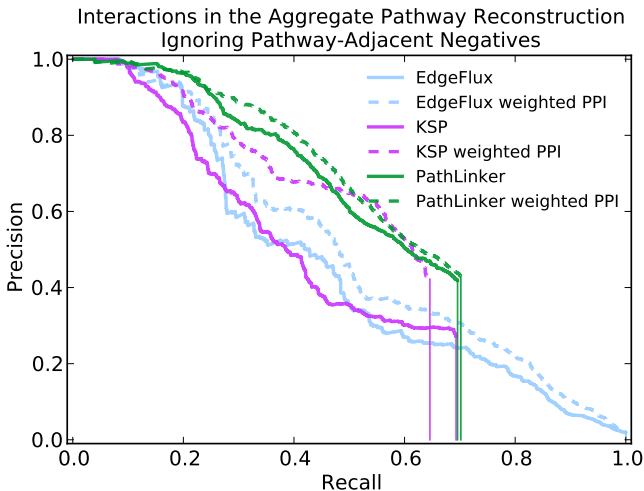


← PathLinker Summary

Recovering Proteins in a Pathway

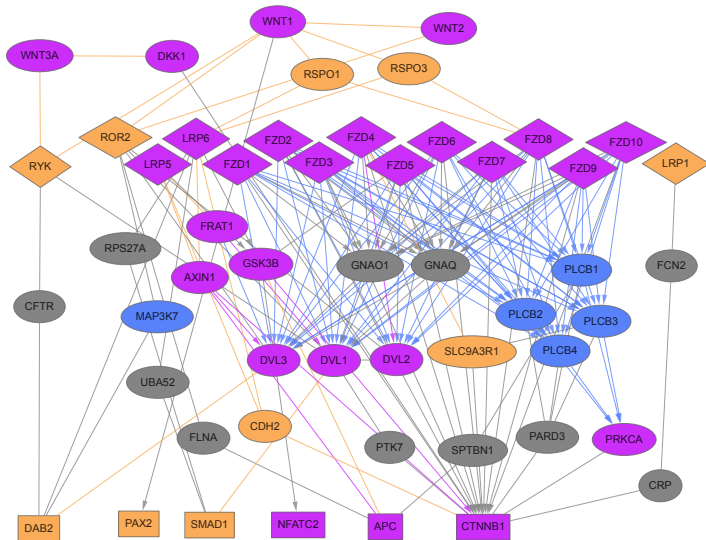


PathLinker on a Weighted PPI

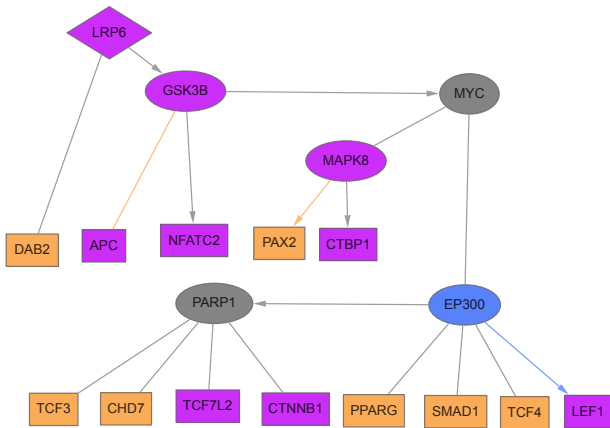


► PathLinker Summary

PathLinker Network

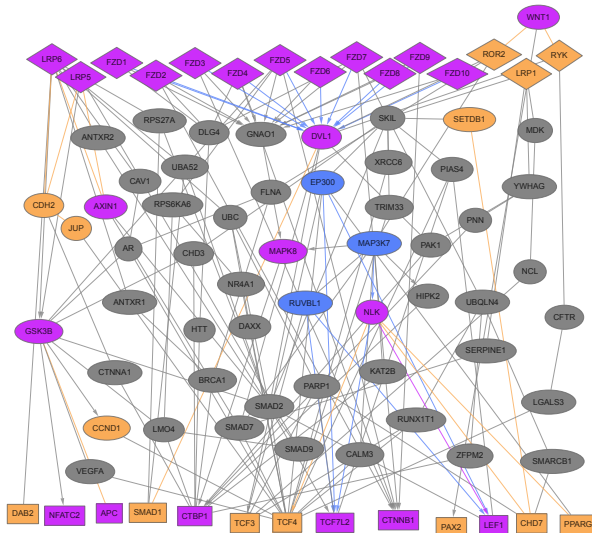


PCSF Network



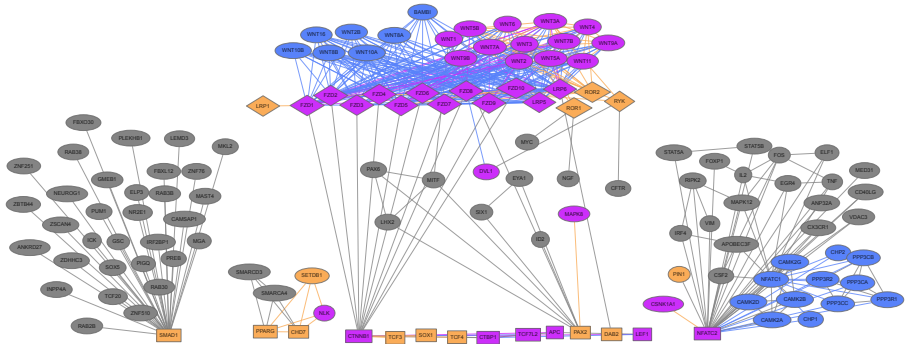
► PathLinker Network

ANAT Network



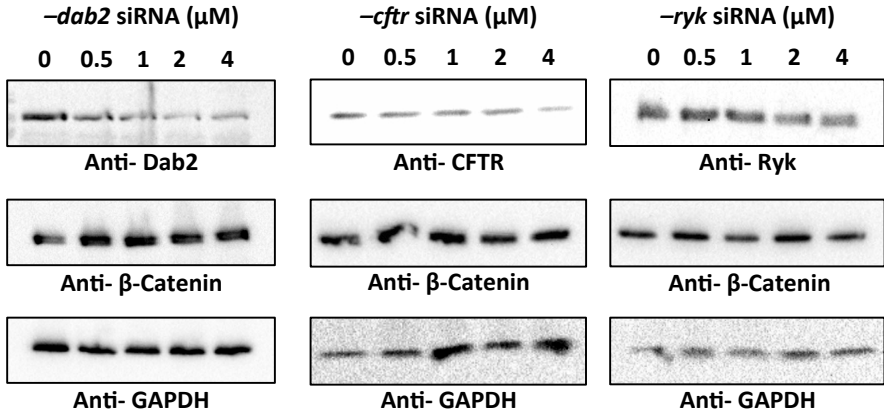
▶ PathLinker Network

IPA Network



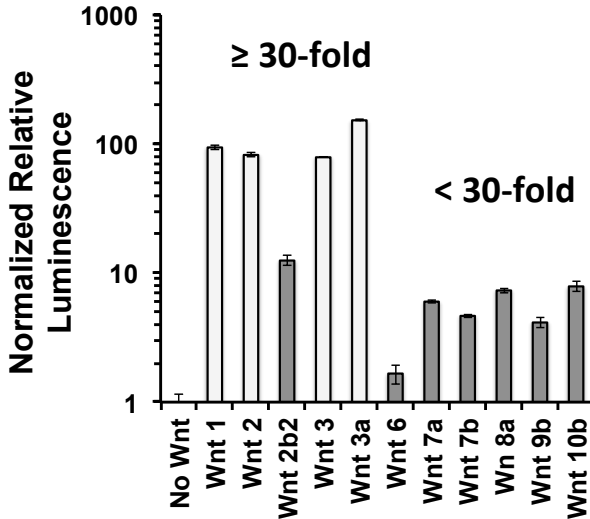
► PathLinker Network

Luciferase Reporter Efficacy



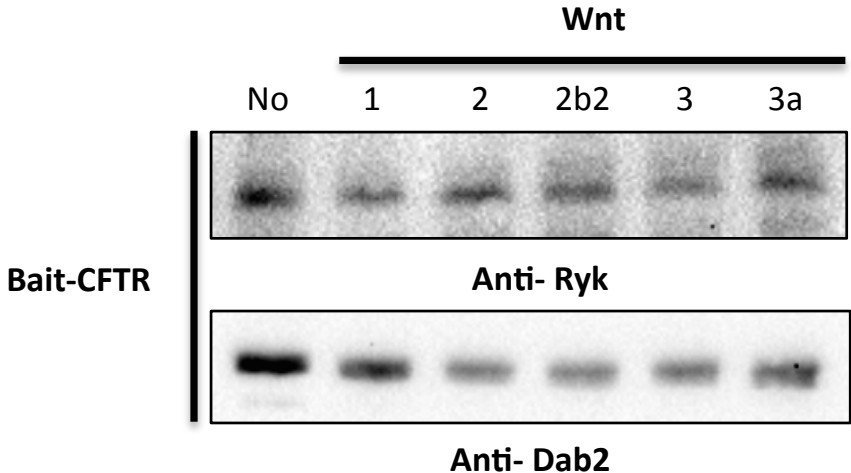
▶ siRNA Experiments

siRNA Silencing Efficacy



► sWnt3a Experiments

Co-Immunoprecipitation Experiments



► sWnt3a Experiments