Functional Significance Checking in Biological Networks: Theory and Implementation

PhD Defence of

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Department of CSE
IIT Bombay

September 2020

Typical Molecular Cell Biology Experiment

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Hypothesis formation

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Reagents, cells, ...

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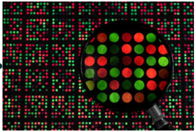
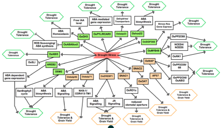


μarray/RNA-Seq

Typical Molecular Cell Biology Experiment

Hypothesis formation

Domain knowledge



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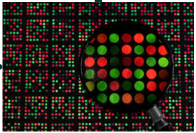

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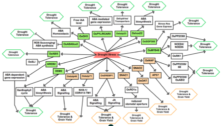

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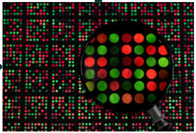

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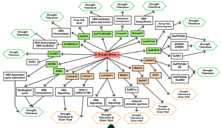
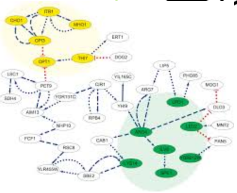
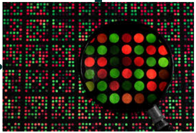




Explanation!

Reagents, cells, ...



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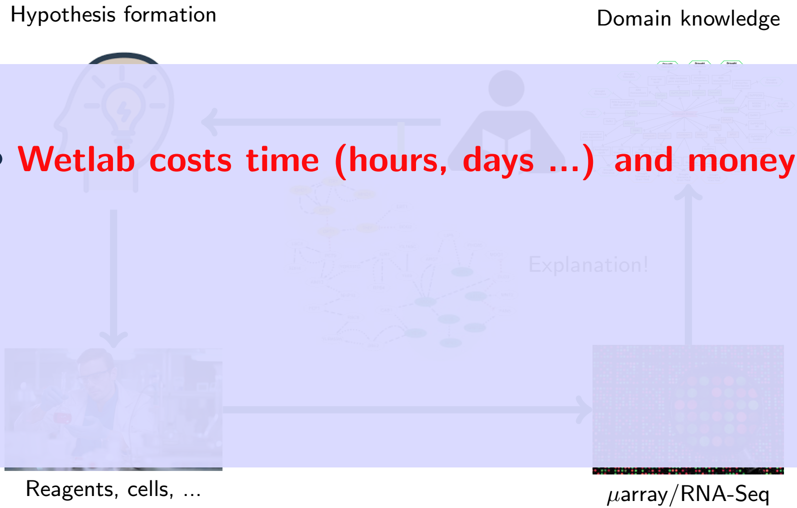
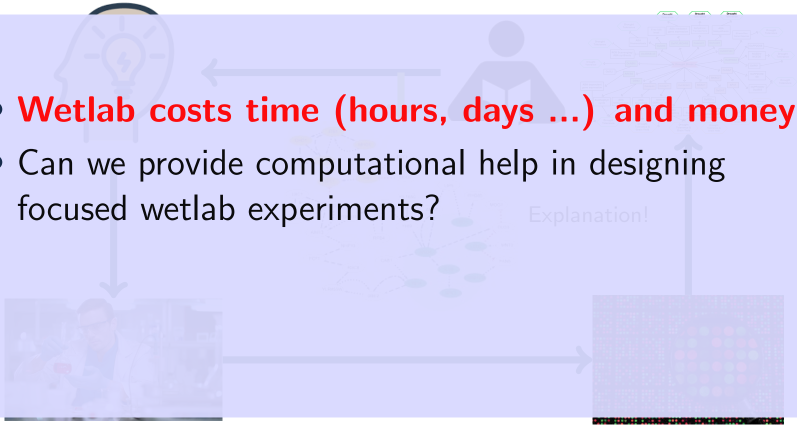


- **Wetlab costs time (hours, days ...) and money**

Typical Molecular Cell Biology Experiment



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- Can we provide computational help in designing focused wetlab experiments?

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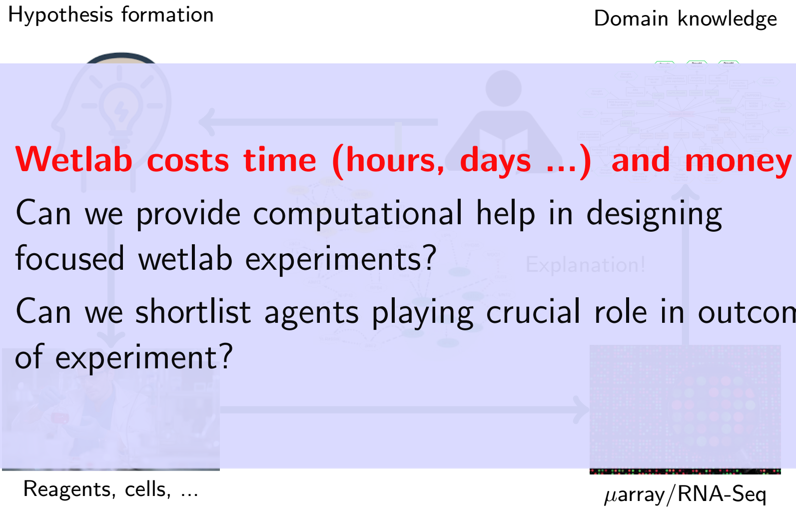

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- Can we shortlist agents playing crucial role in outcome of experiment?

Molecular Biology: A Quick Primer

- Cell, nucleus, chromosome, DNA
- Gene: Part of DNA encoding a small set of functions

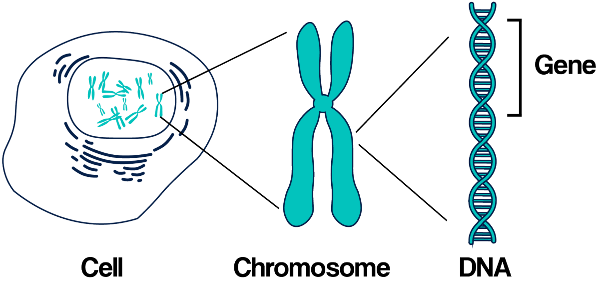

Image source: kintalk.org

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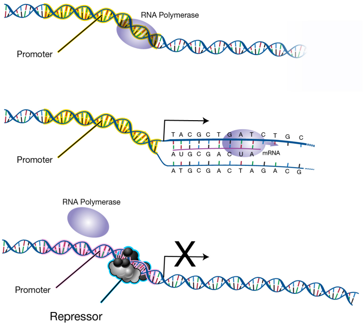

Image source: genome.gov

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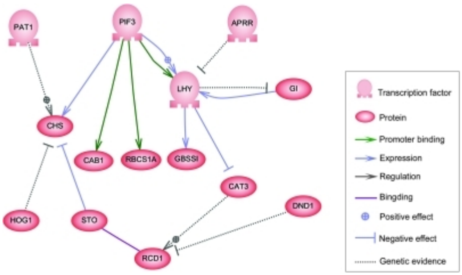

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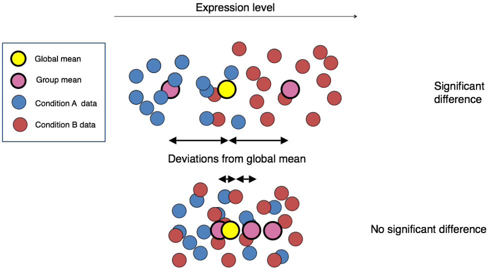

Image source: hbctraining.github.io

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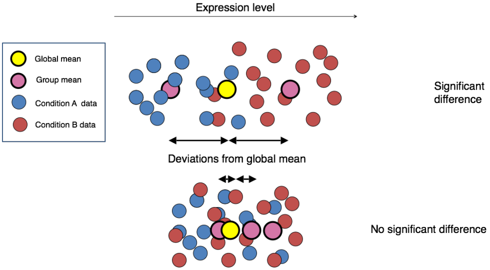
Not differentially expressed $\neq$ Not expressed/repressed



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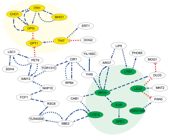
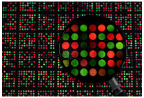
What a biologist wants

Domain Knowledge as Graph





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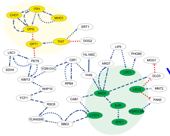
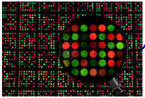


Algorithm ?



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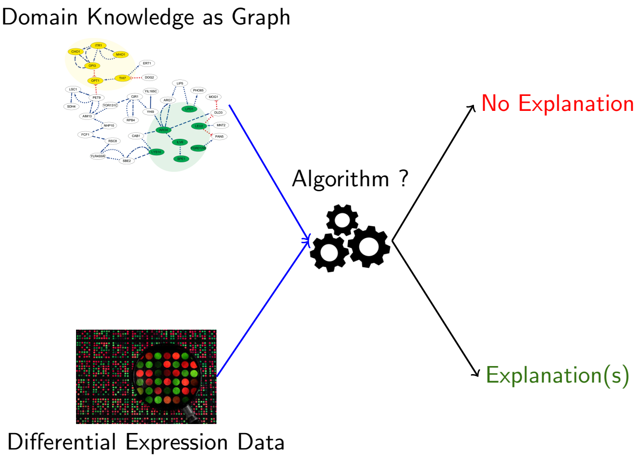

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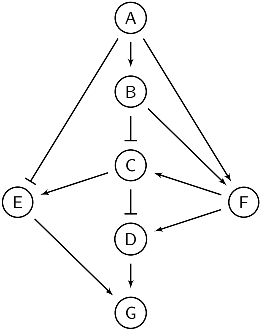
- 7 genes of interest: A, B, C, D, E, F, G



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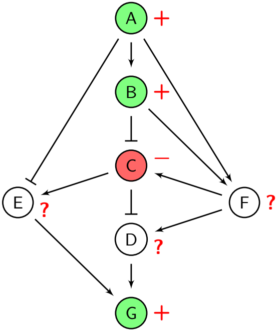

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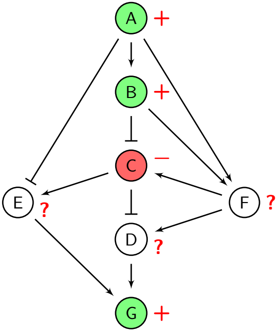

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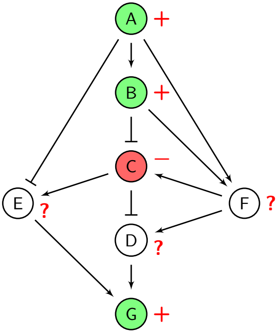

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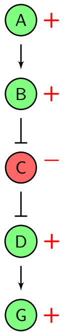

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- **What if D did not play any role?**
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- If all diff. expressions & domain-knowledge encoding is correct
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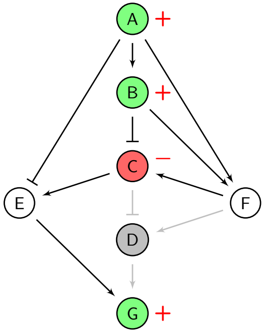

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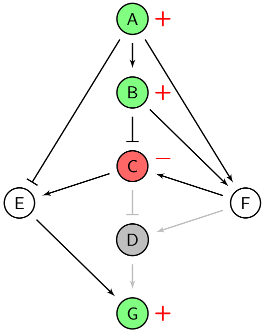

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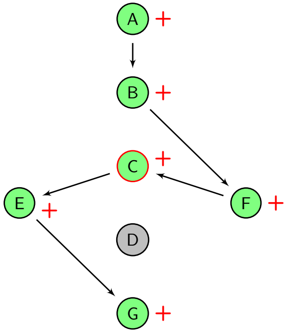

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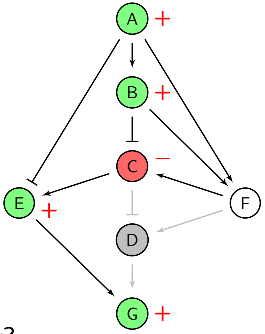

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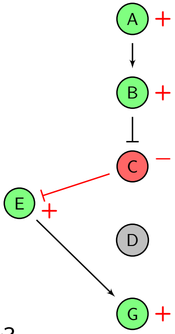
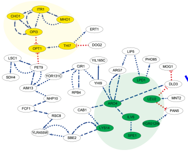


The Biologist Asks for More

Domain Knowledge as Graph

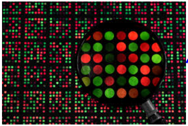


Algorithm

Gene1

Gene2

Gene3



Differential Expression Data

"Functionally significant"

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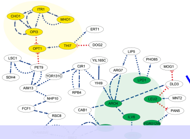
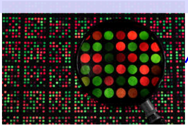


Gene1

Algorithm

Too difficult to solve in practice

Gene3



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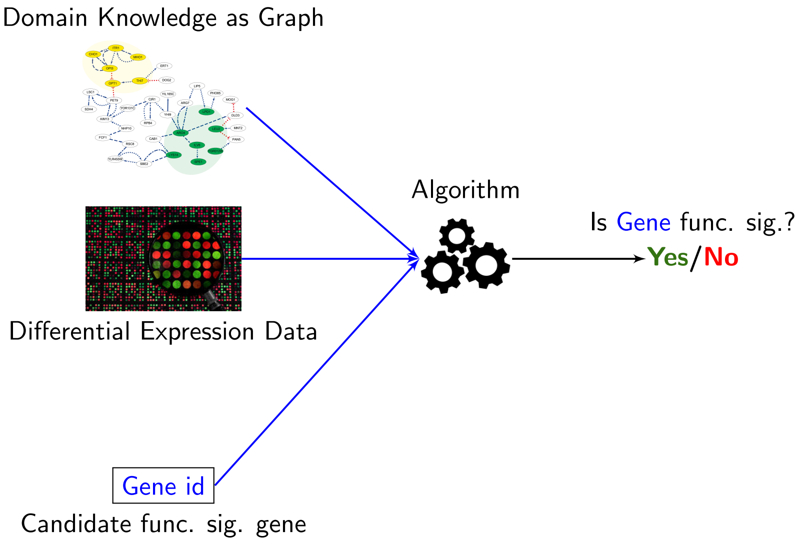
Simplifying Biologist's Ask



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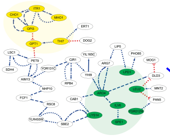
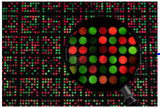


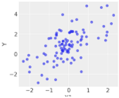


Differential Expression Data

Algorithm

Is Gene func. sig.?

Yes/**No**



Discrete "Error" Bounds

Gene id

Candidate func. sig. gene

Constraint-solving based earlier work for bio-explanations

- Answer Set Programming
  - Gebser et al.[2010, 2011]
- Influence graph and sign consistency with SAT/MaxSAT formulation
  - Soulé [2006], Segel et al. [2006], Guerra and Lynce [2012]
- Other constraint solving techniques
  - Yeang et al. [2004, 2005], Guziolowski et al. [2007], Ourfali et al. [2007], Chasman et al. [2014], Markowetz et al. [2007]
- SAT-based encodings
  - Sharan and Karp [2012, 2013], Dunn et al. [2014, 2017], Shavit et al. [2015], Yordanov et al. [2016]

Contributions of thesis

Three main contributions:

- Problem formulation
- Complexity characterization
- Practical algorithms, implementation, experiments

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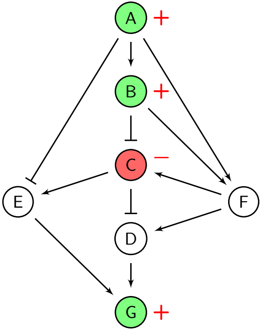
Explanation Subgraph Problem

Given:

- Domain-knowledge graph $G = (V, \mathcal{E}, \mu)$, where $\mu : \mathcal{E} \to \{\text{activation}, \text{inhibition}\}$
- Differential expressions $\lambda : V \to \{+, -, ?\}$
- Stimulus s and target t vertices with $\lambda(s) = +, \lambda(t) = +$



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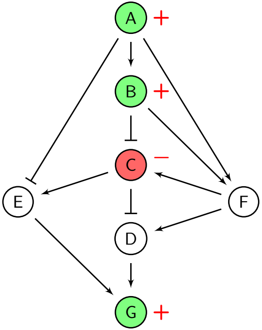
- s, t in G'
- λ' consistent with λ, $\mu' = \mu$
- Every vertex in G' reachable from s
- Every s-t path in G' passes through some $v \notin \{s, t\}$ with $\lambda(v) = +$
- All node and edge labels in G' mutually consistent



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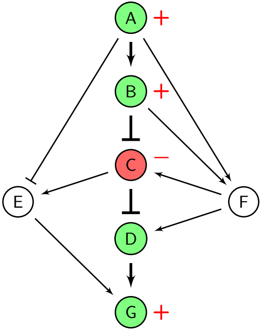

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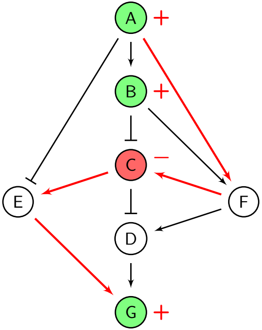

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- Bound n on diff. expr. measurement error, and e on edge label error in domain-knowledge graph

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G' is an (n, e) **relaxed explanation subgraph** for G, λ and R.

Functional Significance Problem

Given:

- Domain-knowledge graph $G = (V, \mathcal{E}, \mu)$, where $\mu : \mathcal{E} \to \{\text{activation}, \text{inhibition}\}$
- Differential expressions $\lambda : V \to \{+, -, ?\}$
- Stimulus s and target t vertices with $\lambda(s) = +, \lambda(t) = +$
- Relaxation weight $R : V \cup \mathcal{E} \to \mathbb{N} \setminus \{0\}$ measuring cost of admitting error in a diff. expr. or edge label
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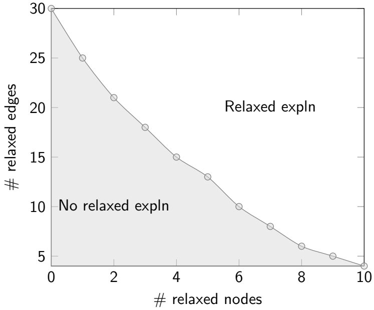
Does removing c force us to admit more errors in finding an explanation subgraph?

Structure of Error (Relaxation) Window

- Explanation region: Relaxed explanation exists
- Non-explanation region: No relaxed explanation exists



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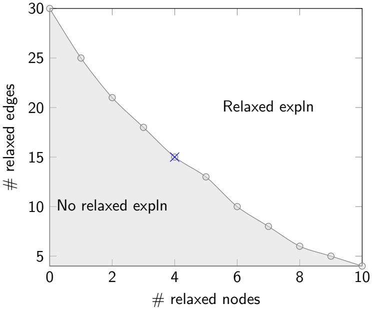

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Functional Significance: Intuition

- Assumptions
  - A1: True explanation corresponds to point within relaxation window
  - A2: True explanation at a Pareto-optimal point $(n^\star, e^\star)$

Under A1, A2, a node v is *functionally significant* if its removal from G leaves no $(n^\star, e^\star)$-relaxed explanation.

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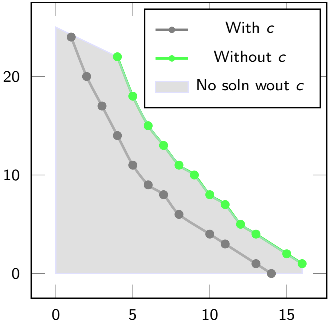
$\boxed{\text{Problem: } (n^\star, e^\star) \text{ not known a priori}}$

Functional Significance through Pareto Dominance

Pareto Dominance

For every (n, e)-relaxed explanation without c, does there exist a **different** (n', e')-relaxed explanation with c, where $n' \leq n$ and $e' \leq e$?

Does Pareto curve with c dominate (below and to left of) Pareto curve without c?



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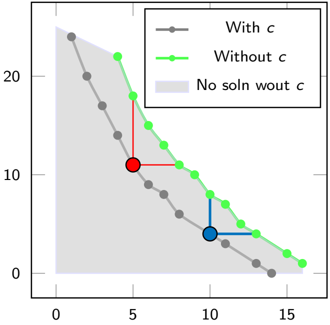

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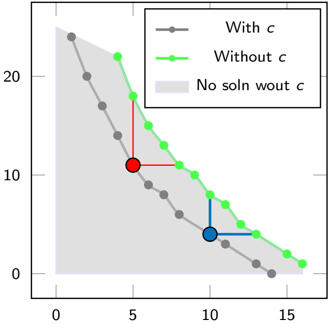

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c is functionally significant if above Pareto dominance holds

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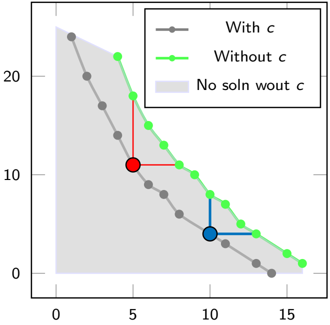

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Function significance checking

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Knowledge of $(n^\star, e^\star)$ not needed

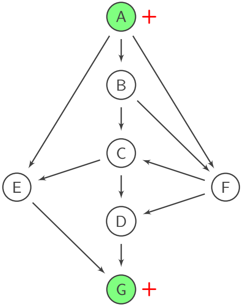
Contributions of thesis

Three main contributions:

- Problem formulation
- $\boxed{\text{Complexity characterization}}$
  - Relaxed explanation finding
  - Functional significance checking
- Practical algorithms and implementation

Features of input graph

Motivated by biological requirements



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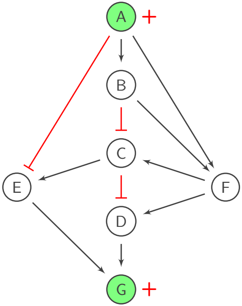
- **Inh:** Presence of inhibition edges



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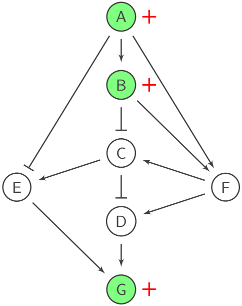

Features of input graph

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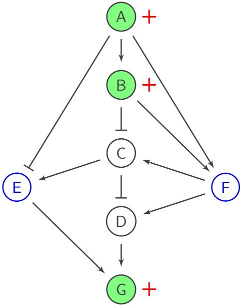

Implication constraints:

- F $\longrightarrow$ E

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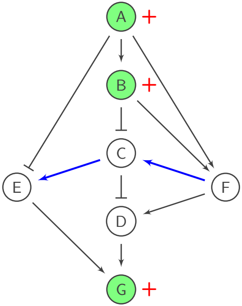

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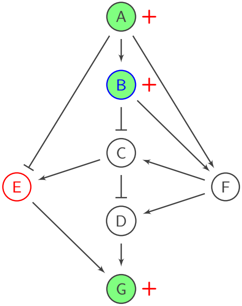

Implication constraints:

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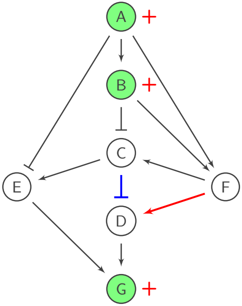

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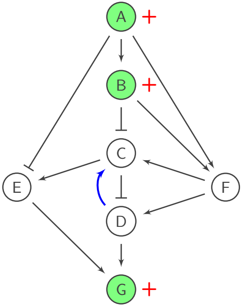
- **Cycles:** Presence of cycles in graph

  From feedback loops in biological pathways



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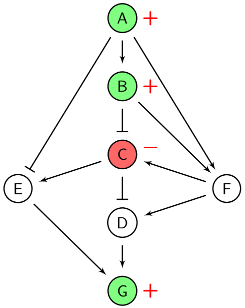
Finding explanations: Why not a graph traversal?

- Topological path not enough
- Must satisfy requirements for explanation subgraph



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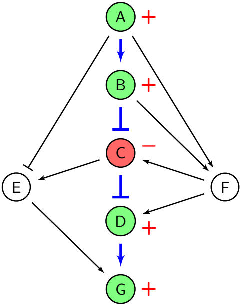
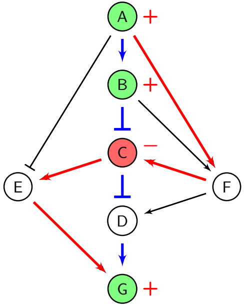


Finding explanations: Why not a graph traversal?

- Topological path not enough
- Must satisfy requirements for explanation subgraph
- Blue path is an explanation
- Red path is not an explanation
  - No $+/-$ assignment to E, F works
  - Path doesn't pass through B



Relaxed explanation finding complexity

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  - No edges/nodes can be relaxed
- Simply having non-$(0, 0)$ relaxation bounds doesn't make things easier
- Focus on $(0, 0)$-relaxed explanation finding complexity

$(0, 0)$-relaxed explanation finding complexity

A primary contribution of thesis

Case	Inh	+ Label	+ve NC	+ve EC	-ve NC	-ve EC	Cycles	Complexity
1	Y	Y	-	-	-	-	-	NPC
2	Y	-	Y	-	-	-	-	NPC
3	Y	-	-	Y	-	-	-	NPC
4	Y	N	N	N	N	N	N	P
5	Y	-	-	-	-	-	Y	NPC
6	-	-	-	-	Y	-	-	NPC
7	-	-	-	-	-	Y	-	NPC
8	-	Y	-	-	-	-	Y	NPC
9	-	Y	-	Y	-	-	-	NPC
10	N	Y	-	N	N	N	N	P
11	N	N	-	-	N	N	-	P

- Reduction from 3-SAT in cases 1,2,3,6,7,9
- Reduction from even-length path in case 5
- Reduction from s-t path through p in case 8

$(0,0)$-relaxed explanation finding complexity

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Case 1: Inhibition edges, +ve internal labels

- NP-hardness by reduction from 3-SAT

$$\varphi_1 = (x_1 \vee \neg x_2 \vee x_3) \wedge (\neg x_3 \vee x_4 \vee \neg x_1) \wedge (x_2 \vee \neg x_3 \vee x_4)$$

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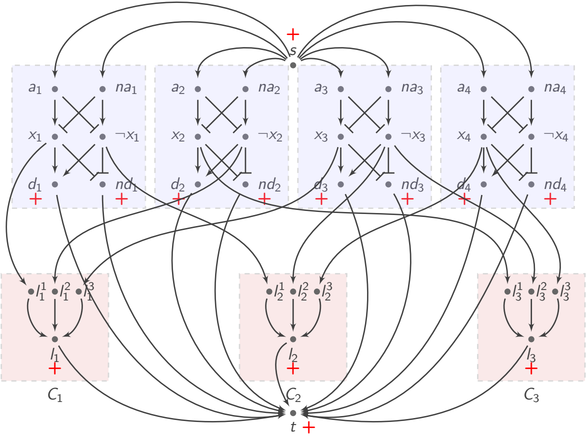

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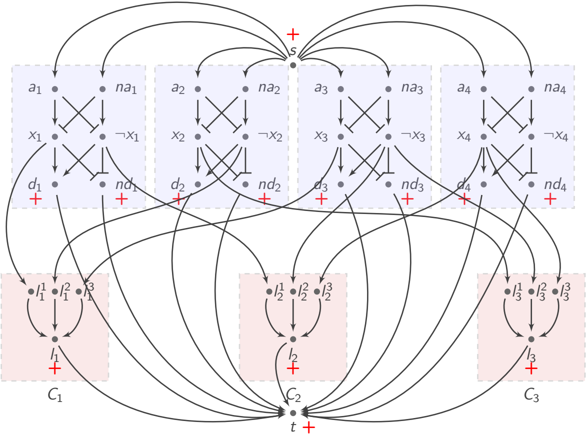
- Variable property
  - d_i, nd_i as $+$
  - ensures exactly one of x_i and $\neg x_i$ is $+$ other one is $-$



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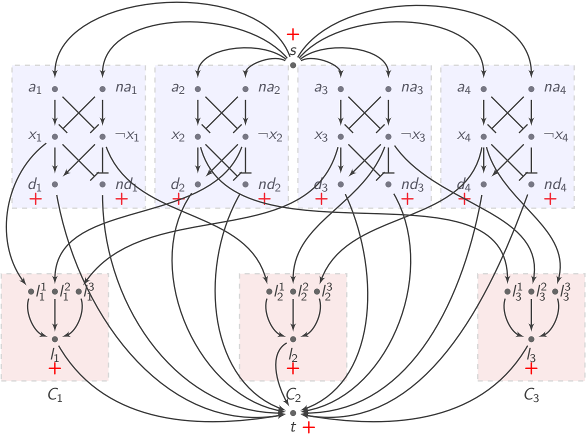

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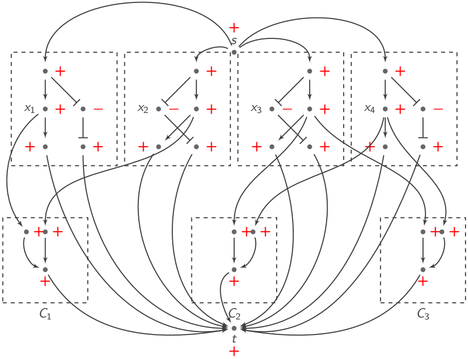

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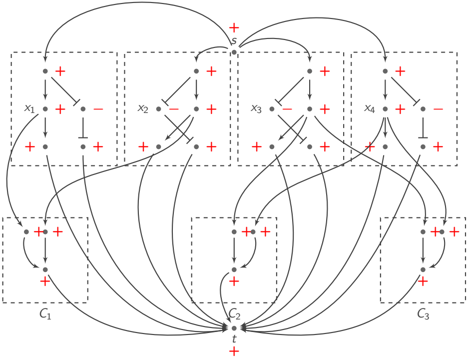
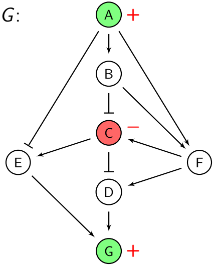


- Similarly, from every $(0,0)$-relaxed expln, a satisfying assignment can be constructed

Case 4: Acyclic graph with inhibition edges

G:



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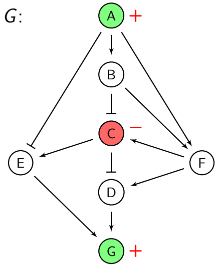

$\widehat{G}$:

- Build a modified graph $\widehat{G}$ from given graph G
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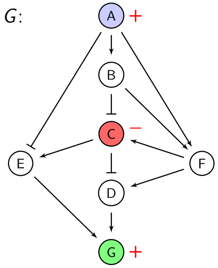
Case 4: Acyclic graph with inhibition edges



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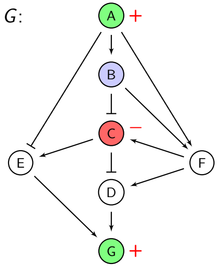

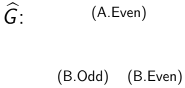

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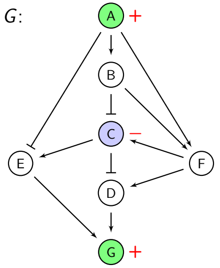

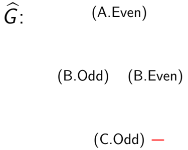

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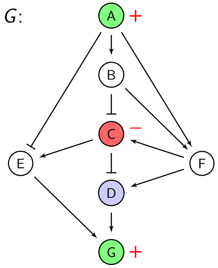

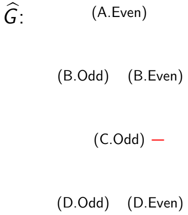

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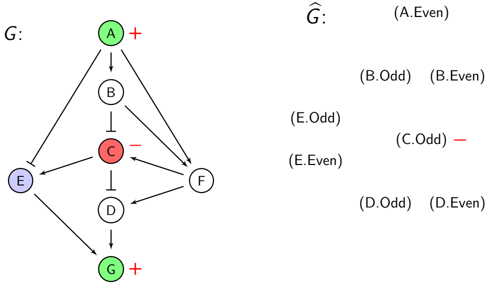

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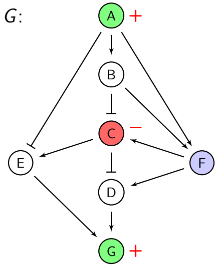

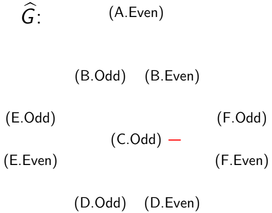

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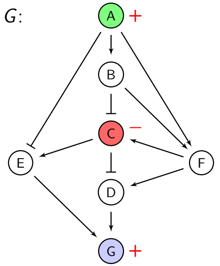

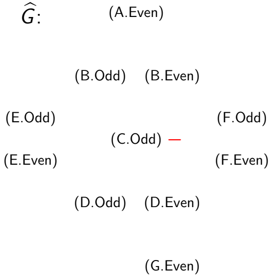

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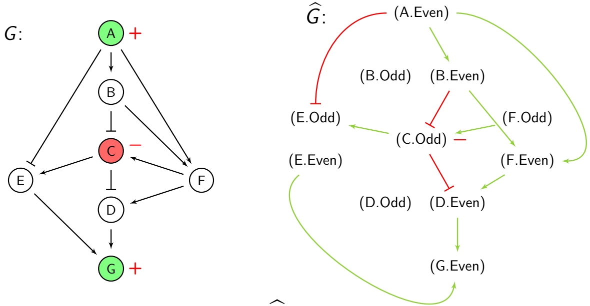

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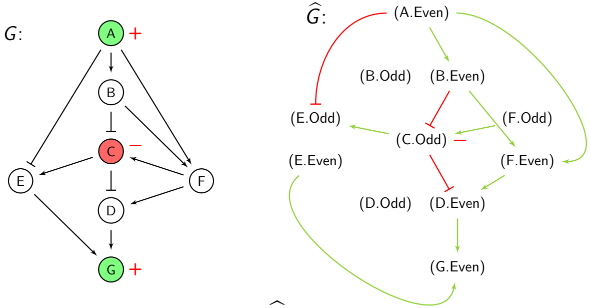

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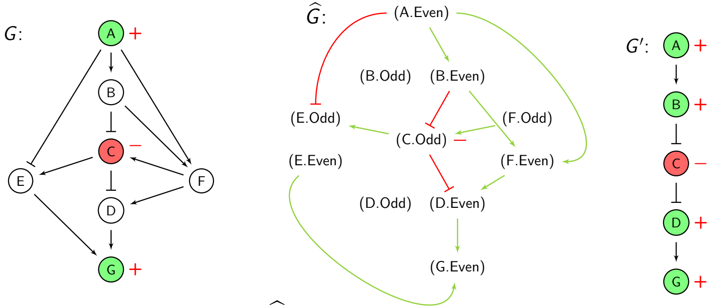

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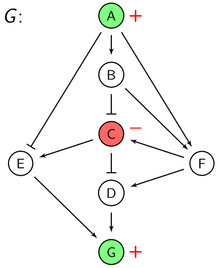

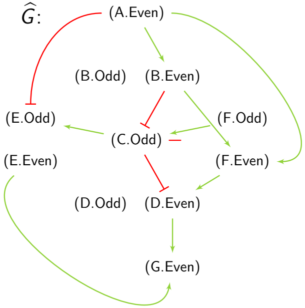

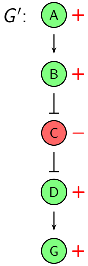

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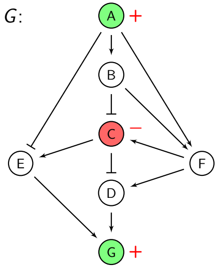

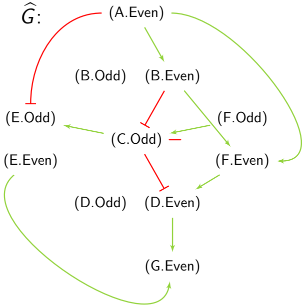

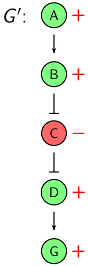

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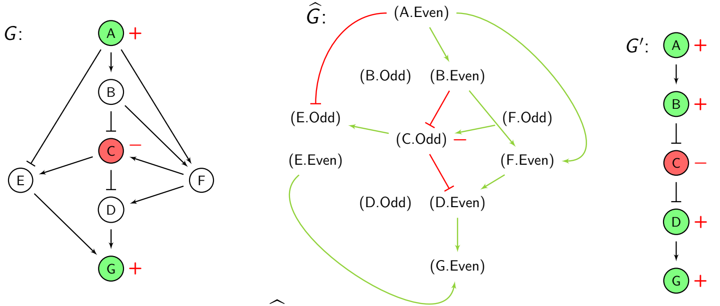

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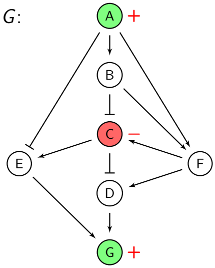

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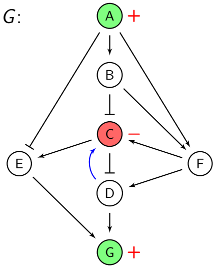

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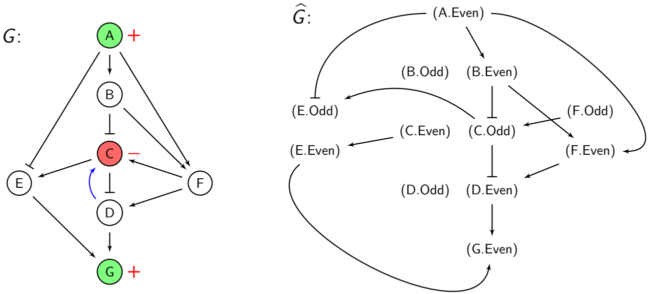

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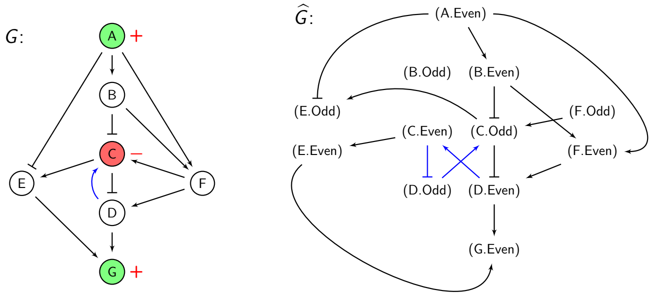

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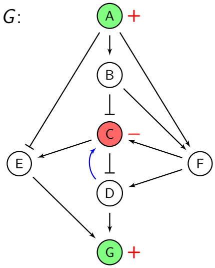

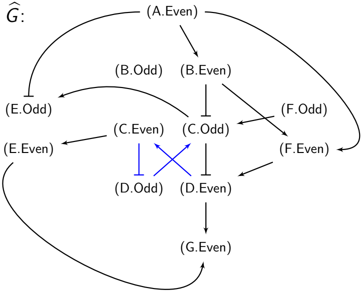

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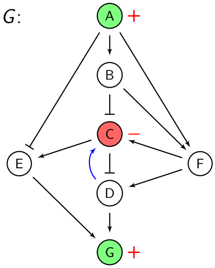

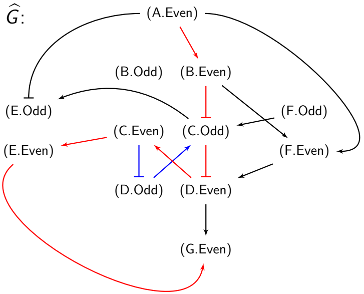

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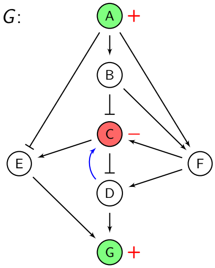

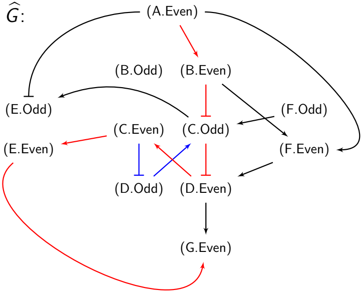

- For some nodes both Odd and Even states exist on s-t path
- Not every path corresponds to a consistent labeling
- Previous algorithm cannot work if there are cycles with odd no. of inhibiting edges

Case 5: Cyclic graph with inhibition edges

- Reduction from even-length path finding problem
- *Lapaugh and Papadmitriou, 1984*: Given a directed graph it is NP-complete to decide if there is an even-length simple path from source to target
- Turns out that $\boxed{\text{Case 5 is NP-hard}}$

Functional significance checking (FSC) complexity

Given:

- Labeled graph encoding domain-knowledge
- Differential expressions
- Stimulus s and target t
- Relaxation weight function
- Candidate functionally significant node c

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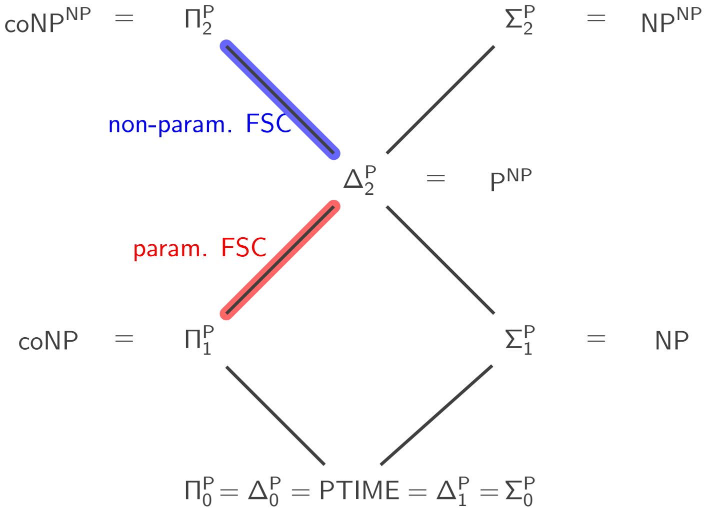
Locating FSC in the Polynomial Hierarchy



Contributions of thesis

Three main contributions:

- Problem formulation
- Complexity characterization
- $\boxed{\text{Practical algorithms, implementation, experiments}}$
  - Complexity results justify making polynomially many SAT solver calls
  - Experiments on synthetic and real benchmarks
  - Validation from wet-lab experiments

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- Very long paths may not be meaningful in biological contexts

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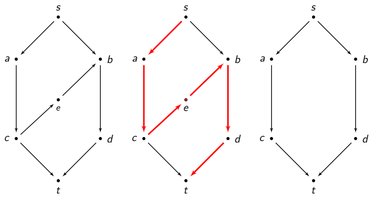
- Remove nodes/edges present *only* in s-t paths longer than given bound (Δ)

$\Delta = 3$



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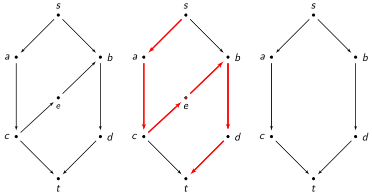

- Some s-t paths may still be longer than Δ.

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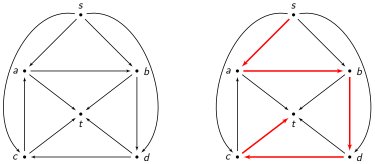

- Every node/edge in such path also on some s-t path of length $\leq \Delta$

Encoding relaxed explanation finding as SAT

Primary Boolean variables:

- For each vertex v in graph
  - p_v: is v present in explanation?
  - a_v: is v active in explanation?
  - r_v: is v relaxed?
  - $d_{v,0}, \ldots d_{v,\log_2 \Delta}$: variables encoding (shortest) distance from s to v in explanation
- For each edge (u, v) in graph
  - $p_{(u,v)}$: is (u, v) present in explanation?
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$$(4 + \log_2 \Delta) \cdot |V| + 3 \cdot |\mathcal{E}| \text{ variables}$$

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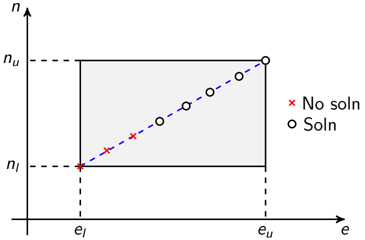
Correctness of encoding

φ^* satisfiable iff there is an (n, e)-relaxed explanation

Efficient computation of Pareto-optimal (PO) curves

Given a relaxation window, find PO curve for existence of relaxed explanation within window

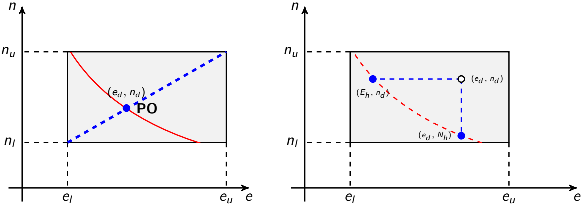


Property of relaxation window

If there is no solution at a specific point on diagonal of window, then there is no solution at any dominating (lower and left) point along diagonal.

Efficient computation of PO curves

- From points on diagonal to PO points



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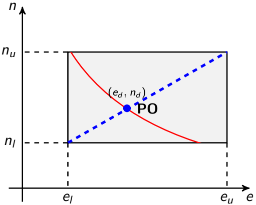
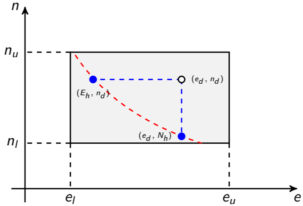
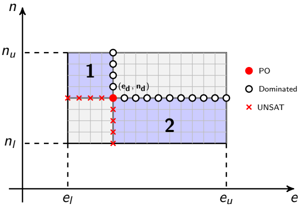
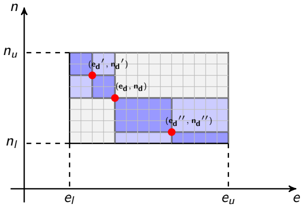




- Recursive method for PO-curve computation





  - $\mathcal{O}(k \cdot \log_2 k)$ calls to SAT solver, where $k = \max(n_u - n_l, e_u - e_l)$

Functional significance checking tool

- Implemented in C++ using Z3 as backend solver
- Inputs:
  - Domain knowledge: pathways (163) from KEGG database
    - Publicly available in XML variant
  - Microarray differential expression data
    - Biologist-provided threshold to decide $+/-/$not differentially expressed
  - Stimulus and target identities
  - Candidate functionally significant actor c
  - Relaxation weights
    - Default: 1 for each node/edge relaxation
  - Bound on path length
  - Node, edge relaxation window
- Outputs:
  - Pareto curves with and without c present
    - Finer-grained info than yes/no answer
  - Relaxed explanation(s): one or as many desired
  - Set of relaxed nodes and edges in an explanation

Experiments

Compute platform:

- Intel(R)-Core(TM)-i7-3770 CPU with 8 cores
- 3.4 GHz, 32 GB RAM

Benchmarks:

- Synthetic
  - Problem instances obtained from reductions used in complexity analysis
    - Can easily build instances of arbitrary size
    - Ground truth (functional significance) known a priori
    - Useful for tool validation
- Real-world
  - From cell-biology experiments by our collaborators
  - Instance size determined by KEGG and path-length bound
  - Ground truth (functional significance) not known a priori
  - Useful for evaluating real utility of our work

Results on synthetic benchmarks

- All benchmarks: inhibition edges, acyclic, internal nodes labeled $+/-$
- Path length bound: max path length in graph
- Each experiment: construct two PO curves (with and w/o candidate func. sig. actor), check separation of curves

Expt	#Vertices	#Edges	Pareto shift (Y/N)	# SAT Calls	Time (in hrs)
Toy-example	7	10	Y	5	.001
Synthetic-5*var*-$W(5,5)$	773	1541	Y	5	0.035
Synthetic-10*var*-$W(5,5)$	1323	2641	Y	5	0.12
Synthetic-12*var*-$W(6,6)$	1823	3641	Y	6	0.36
Synthetic-15*var*-$W(5,5)$	1873	3741	Y	6	0.35
Synthetic-45*var*-$W(0,0)$	473	941	Y	2	0.004

Example Real Benchmark

- Source: TNFα; Target: IKBa (understanding cancer cell signaling)
- 154 pathways from KEGG merged to obtain initial graph
  - # Vertices: 2469; # Edges: 10301
- Path-length bound: 7
  - # Vertices: 296; # Edges: 1896
- Relaxation window: $[0, 30]$ for both nodes and edges

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  - # Vertices: 296; # Edges: 1896
- Relaxation window: $[0, 30]$ for both nodes and edges
- Functional significance of multiple candidates checked
  - Common PO curve (baseline) with all candidates present

Example Real Benchmark

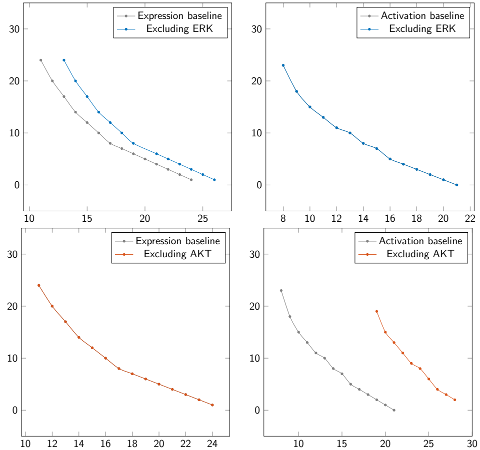
- Source: TNFα; Target: IKBa (understanding cancer cell signaling)
- 154 pathways from KEGG merged to obtain initial graph
  - # Vertices: 2469; # Edges: 10301
- Path-length bound: 7
  - # Vertices: 296; # Edges: 1896
- Relaxation window: $[0, 30]$ for both nodes and edges
- Functional significance of multiple candidates checked
  - Common PO curve (baseline) with all candidates present

	Only "expression" edges to IKBa			Only "activation" edges to IKBa		
FS Cand.	**Pareto shift (Y/N)**	**# SAT calls**	**Time taken**	**Pareto shift (Y/N)**	**# SAT calls**	**Time taken**
Baseline	–	63	9 hrs	–	64	15.6 hrs
ERK	**Y**	63	15 hrs	**N**	64	25.8 hrs
PIK3CD	**N**	68	10.4 hrs	**Y**	64	22.7 hrs
PIK3CA	**N**	68	14 hrs	**Y**	64	18.5 hrs
CREB3	N	57	9.4 hrs	N	57	21.4 hrs
PRKCA	**N**	34	9.8 hrs	**N**	64	32 hrs
TFG	N	47	10 hrs	**N**	65	25 hrs
JNK	**Y**	22	7.2 hrs	**N**	64	34 hrs
RHOA	N	24	9 hrs	**N**	58	21.7 hrs
MAPK10	**Y**	24	11.8 hrs	**N**	64	31 hrs
PLCD3	**Y**	63	35 hrs	**Y**	60	46.8 hrs
P38	N	63	15 hrs	**N**	64	37 hrs
AKT	**N**	68	18.4 hrs	**Y**	54	44 hrs

Time dominated by SAT-solving time

Examples of functional significance checks

Expression plots | Activation plots



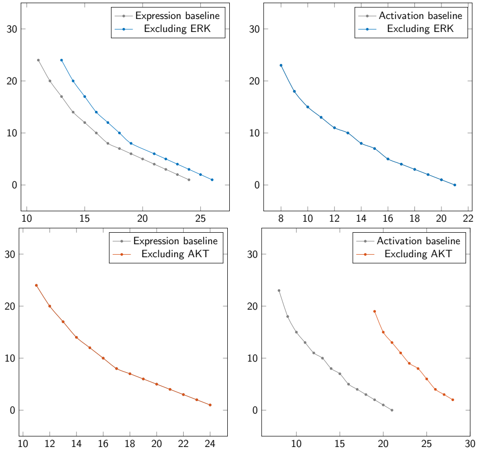
Inference: TNF$\alpha$$-$IKBa signaling

Separation implies functionally significant, under assumptions A1 & A2

Examples of functional significance checks

Expression plots

Activation plots



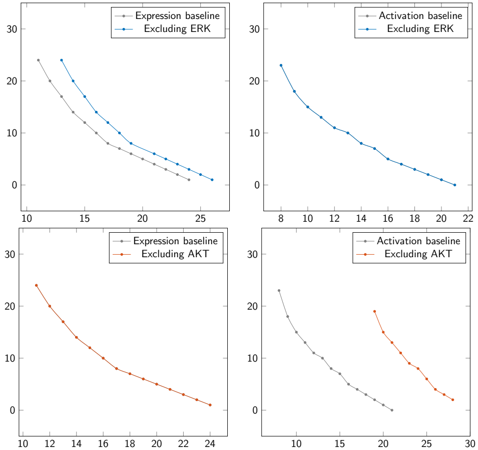
Inference: TNFα−IKBa signaling

- ERK plays role in expression, not necessarily in (in)activation of IKBa

Separation implies functionally significant, under assumptions A1 & A2

Examples of functional significance checks

Expression plots Activation plots



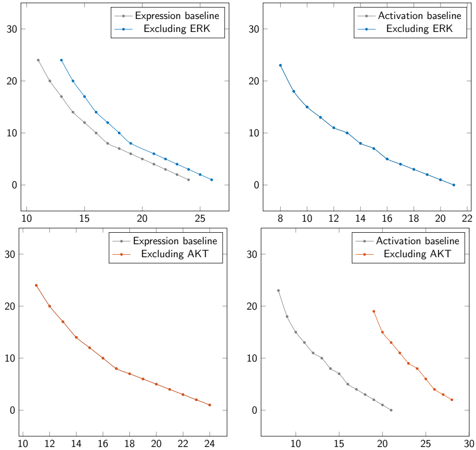
Inference: TNFα–IKBa signaling

- ERK plays role in expression, not necessarily in (in)activation of IKBa
- AKT has opposite role

Separation implies functionally significant, under assumptions A1 & A2

Examples of functional significance checks

Expression plots   Activation plots



Inference: TNF$\alpha$$-$IKBa signaling

- ERK plays role in expression, not necessarily in (in)activation of IKBa
- AKT has opposite role
- Incomplete separation of PO curves
  - Candidate doesn't play role
  - Alternate pathways in absence of candidate
  - Further experiments needed to confirm

Separation implies functionally significant, under assumptions A1 & A2

Wet-lab validation

Wet-lab experiments to validate predictions of functionally significant actors

- Thanks to our biology collaborators
- Computationally func. significant (wet-lab validated):
  - TNFα-IKBa expression: ERK, JNK, MAPK10, PLCD3
  - TNFα-IKBa activation: PIK3CD, PIK3CA, PLCD3, AKT
  - TNFα-A20 expression: ERK
- Other validation experiments yet to be done

Conclusion

- Research motivated by practical problems faced by biologists
- In this work:
  - Formulated relaxed explanation finding and functional significance checking
  - Complexity characterization of problems
  - Developed practical algorithms and tool
  - Experiments on synthetic and real data
    - Predicted functionally significant actors
    - Some predictions validated by wet-lab experiments
- Continuous collaboration with biologists was crucial
  - What assumptions meaningful?
  - What predictions make sense?
  - What can/cannot be wet-lab validated?

Future Work

- Current work binarizes differential expressions ($+$ or $-$)
  - Can we do finer-grained/quantitative analysis?
- Current work abstracts interactions as one-shot (activates or inhibits)
  - Can we incorporate timed dynamics?
- Current work uses single snapshot (microarray data)
  - How to incorporate time series data from same experiment?
  - Extract computationally provable causal relations?
- Can we do a similar analysis with timed and stochastic interpretations of interactions?
- Closing complexity lower/upper bounds of functional significance checking
- Improved encodings for use in tool

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