Network Legos: Building Blocks of Cellular Wiring Diagrams

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August 19, 2008

Goals of Data-Driven Molecular Systems Biology

- Identify the building blocks of wiring diagrams.
- Interconnect the building blocks to build high level models of the cell.
- Understand the interaction of the building blocks over time and under different conditions.

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How do we automatically construct these building blocks?

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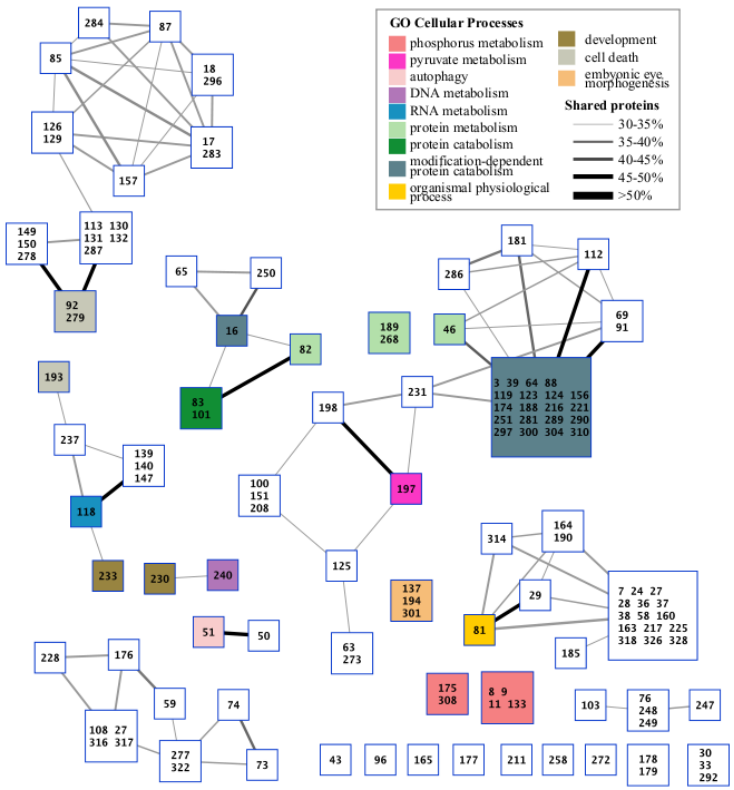

(From Sharan et al., *PNAS*, 2005)

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But cell state is dynamic!

- Active molecular interactions change with time, external signals, and perturbations.
- Decompositions of static and *universal* interactomes may miss many important aspects of cellular activity.
- We must integrate interactomes with dynamic measurements of cell state to compute the cell's response to different conditions.

Molecular Interactomes $\longrightarrow$ Active Networks

- Gene expression data provide dynamic snapshots of cellular activity.
- *Active network*: Molecular interactions activated by the cell in response to a stimulus.
- Methods to integrate interactomes with transcriptional measurements to compute active networks:
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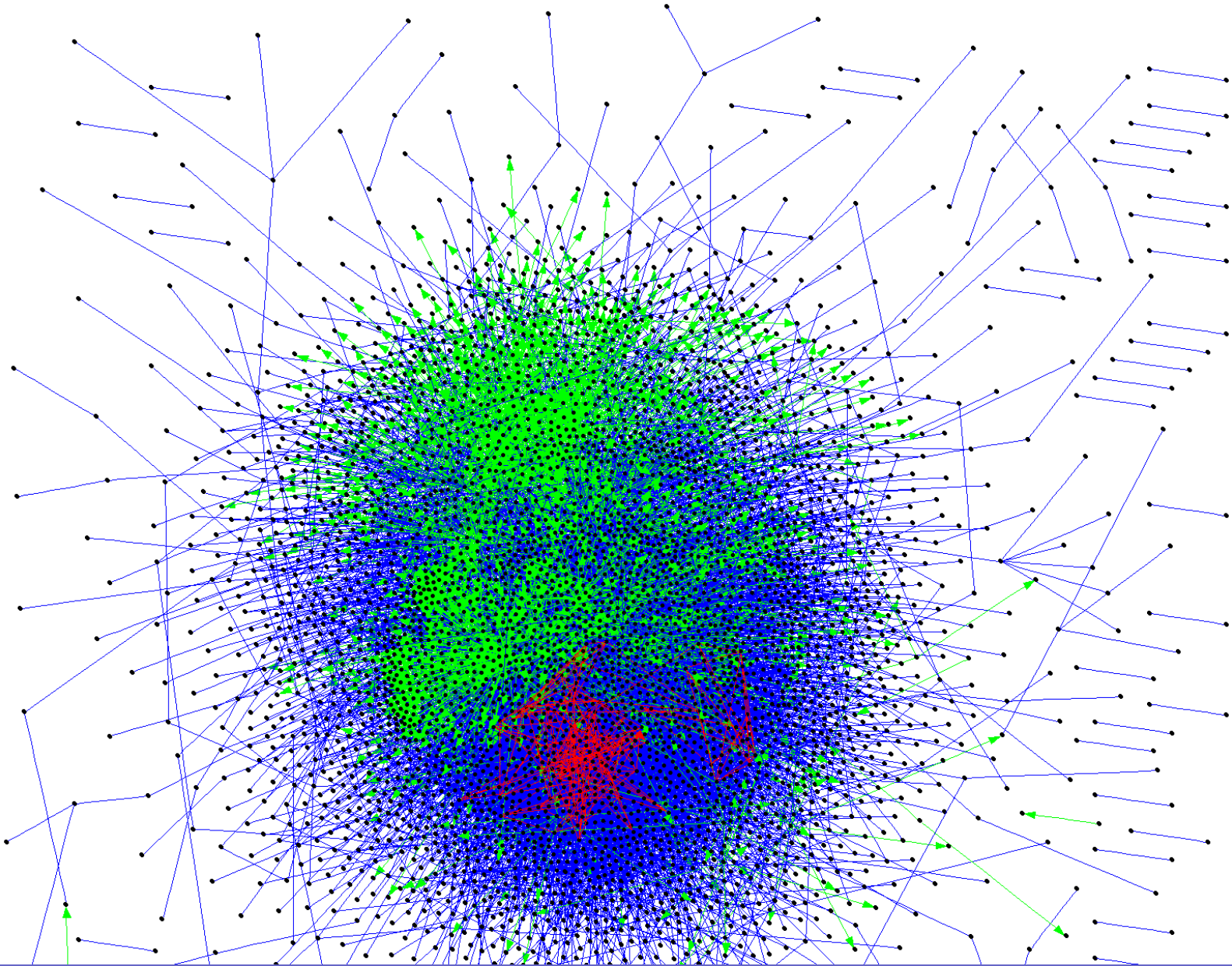

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- These methods usually compute active networks one condition at a time or simultaneously across multiple conditions.

Hypotheses Guiding a New Approach

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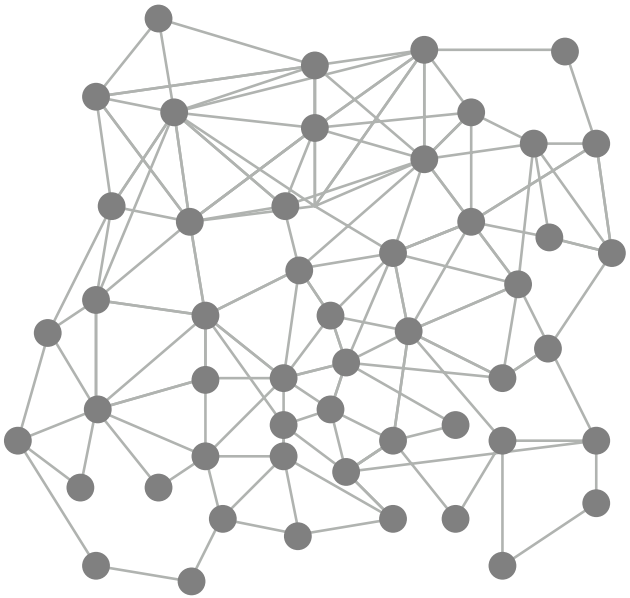
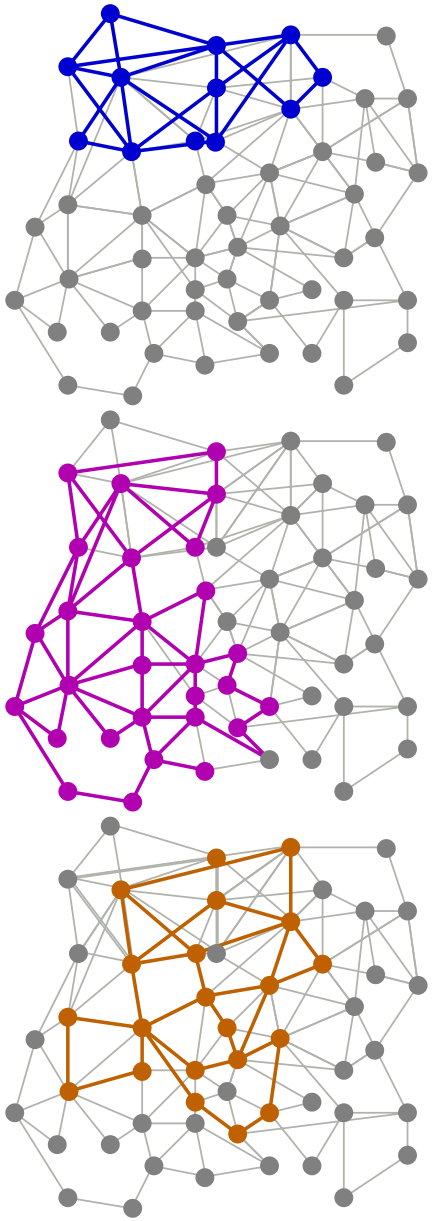
Idea: turn second hypothesis on its head to compute modules from multiple response networks.

Goals of the Network Lego Approach

- Combine active network computation with module detection to compute *network legos*: context-sensitive building blocks of wiring diagrams.
- Potential applications:
  1. Identify pathways uniquely activated in one or more conditions.
  2. Compare and contrast responses of different cell types to the same stress.
  3. Develop a formalism for expressing any active network as a combination of network legos.

Step 1: Molecular Interactome to Active Networks





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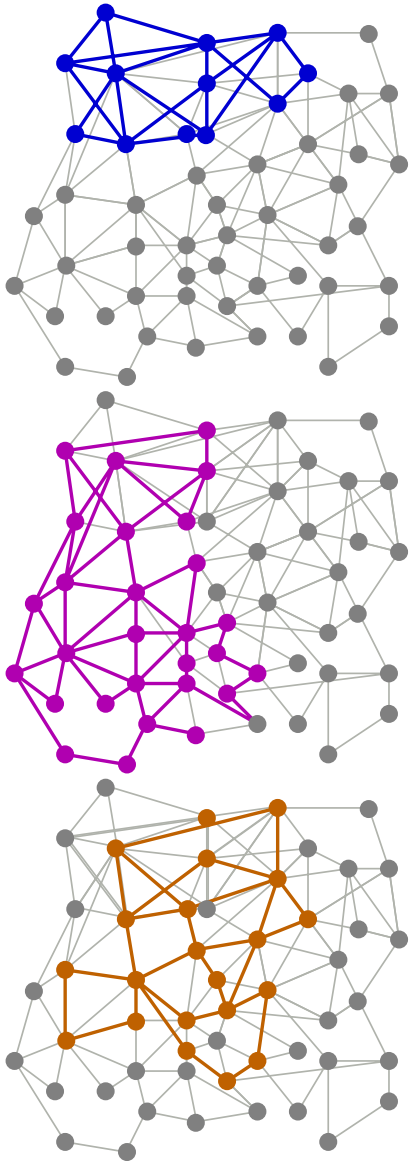

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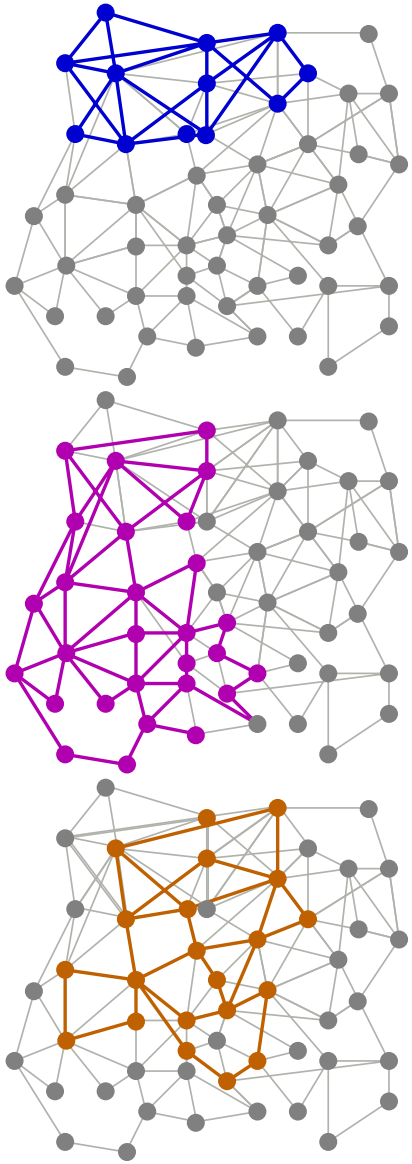

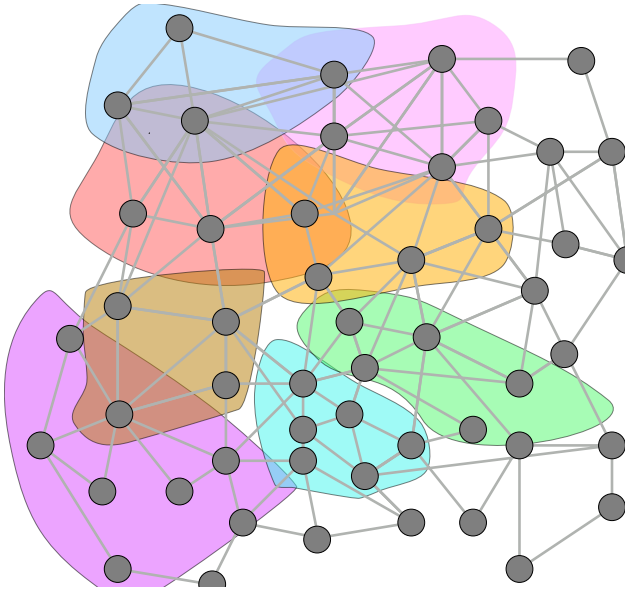

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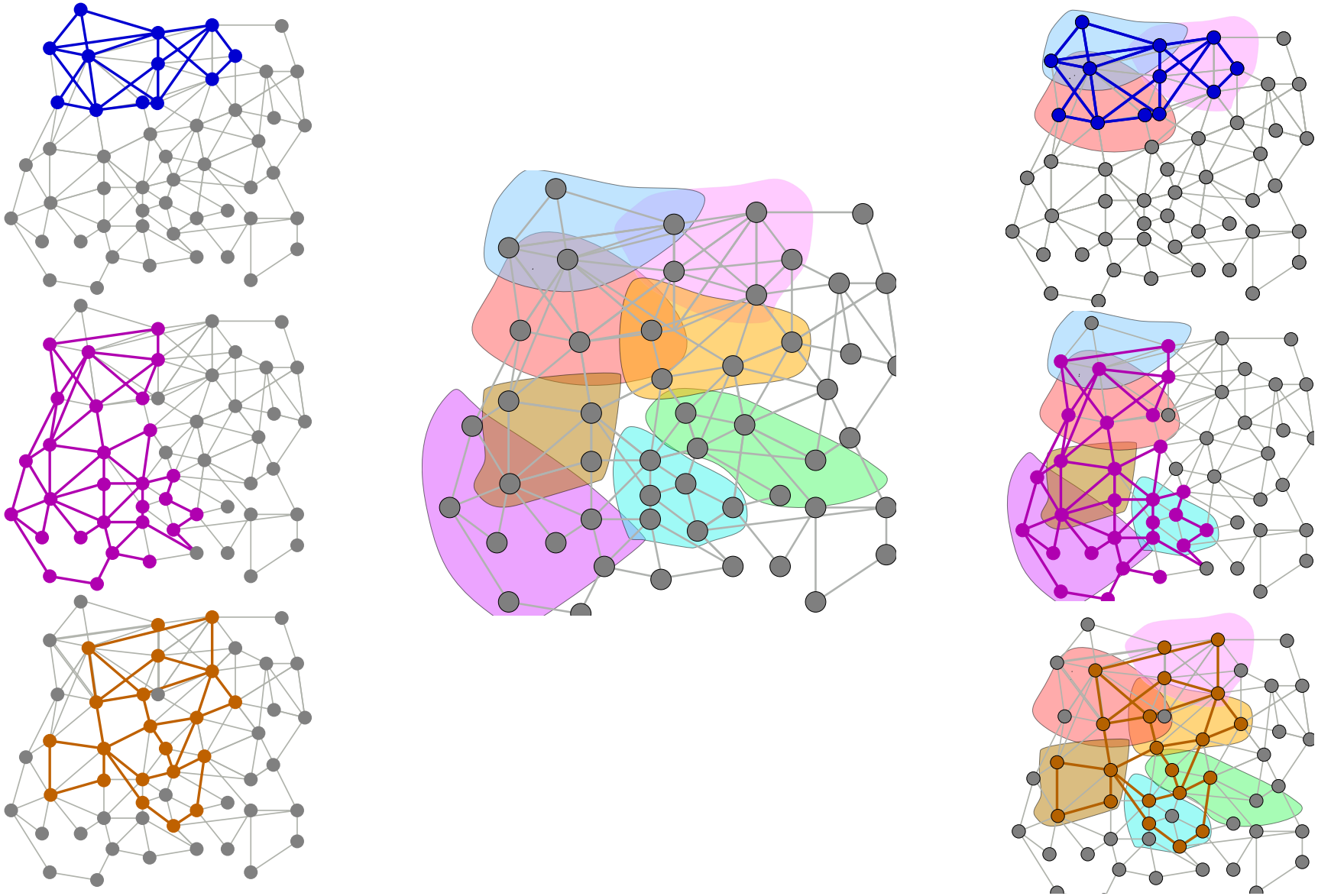

Caveats

- Interactomes are incomplete and noisy.
- Gene expression measurements miss many aspects of cellular state.
- We will consider only presence or absence of an interaction in an active network.
- Network legos are only a mental model of how the cell may operate.

Network Blocks

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- Compute the active network for each condition.
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- A *block* is a triple $(G, \mathcal{I}, \mathcal{E})$ where
  - $\mathcal{I} \subseteq \mathcal{A}$, $\mathcal{I}$ is non-empty.
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  - $\mathcal{I}$ and $\mathcal{E}$ are inclusion-maximal
  - G is a network where each interaction
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ALL, AML, and MLL

- Acute Lymphoblastic Leukaemia (ALL) and Acute Myeloid Leukaemia (AML) are two types of leukaemia.
- Armstrong et al., Nature Genetics 2003 argued that translocations in the Mixed Lineage Leukaemia (MLL) gene identify a disease distinct from ALL and AML.

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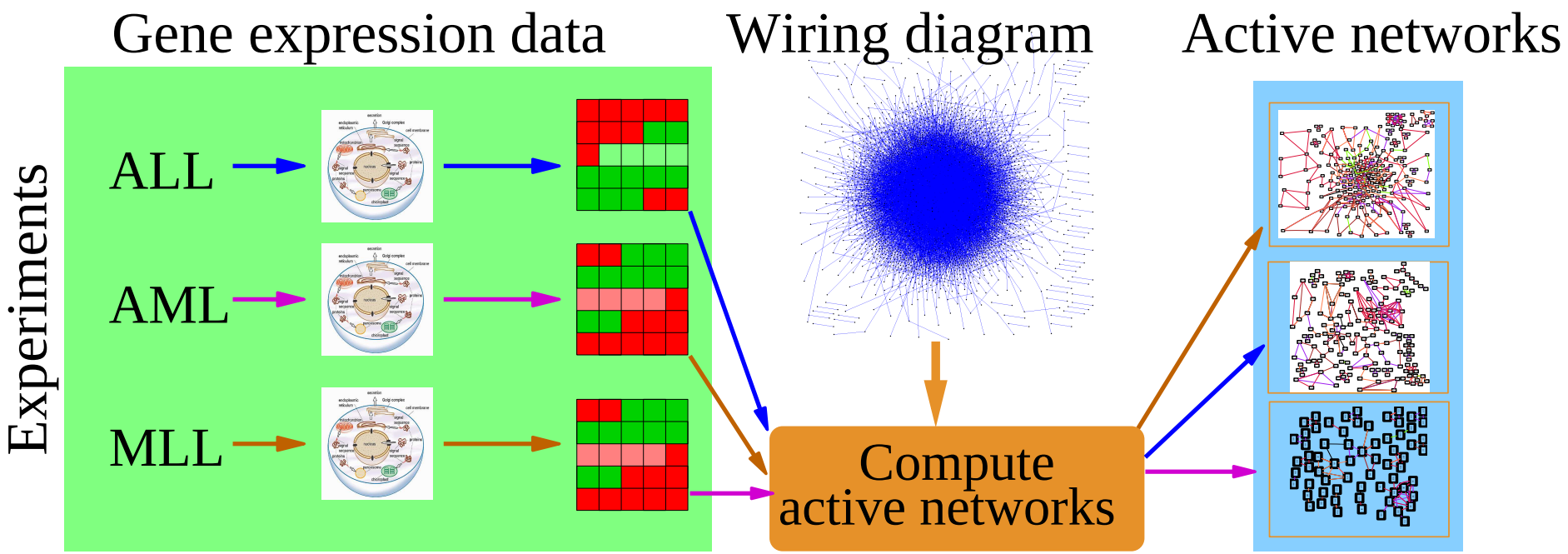
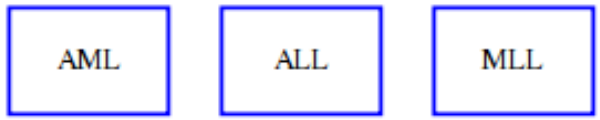
Can we compare active networks to identify subsets of interactions differentially activated in each leukaemia?

Comparing ALL, AML, and MLL



Computing 17 Comparisons



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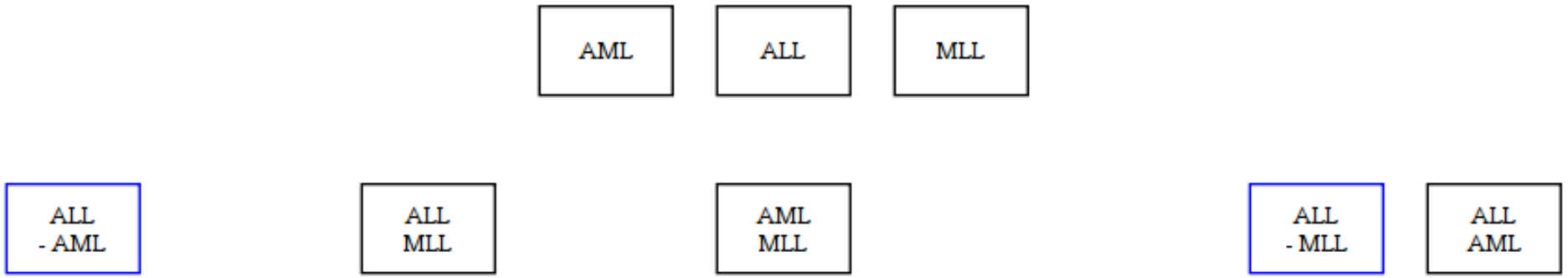

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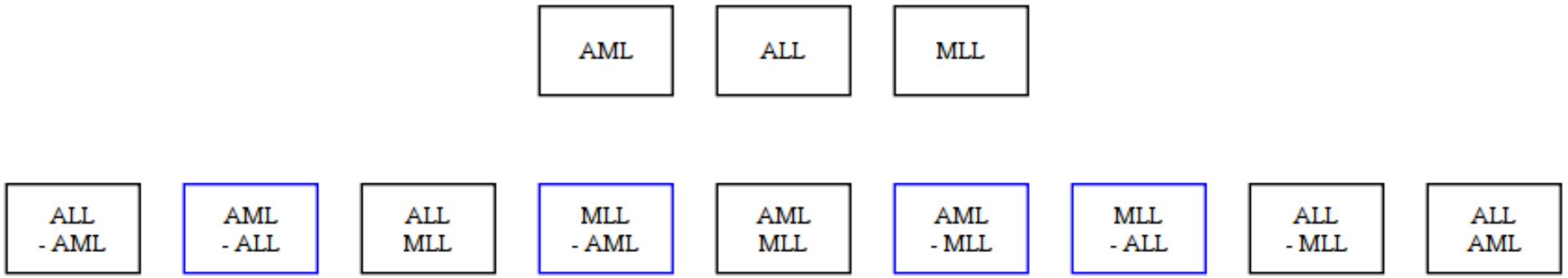

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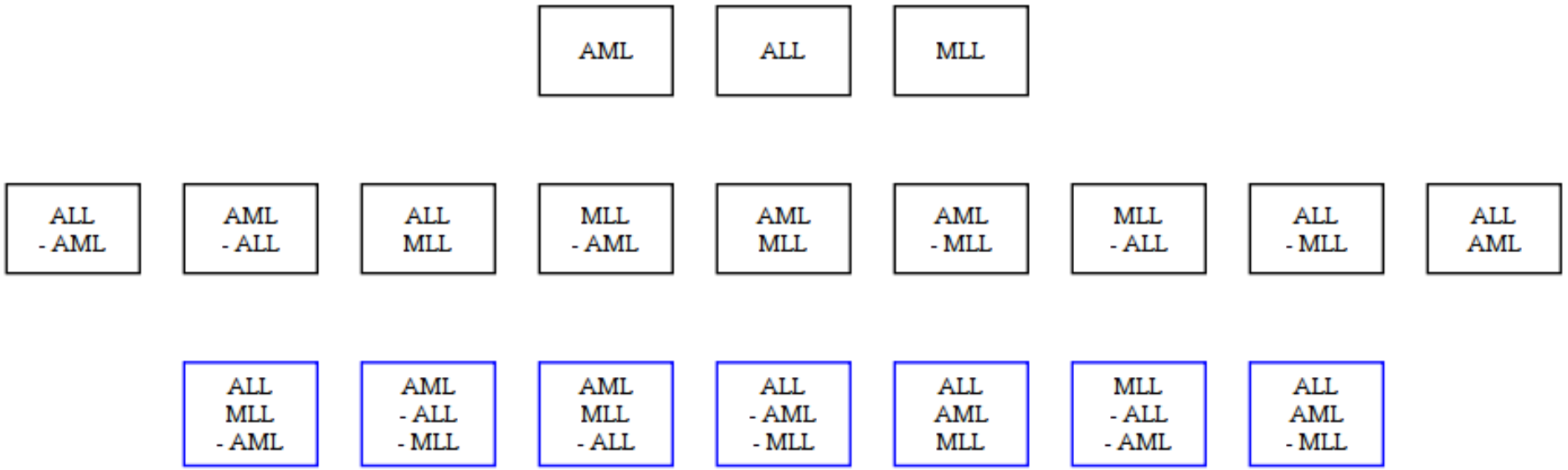

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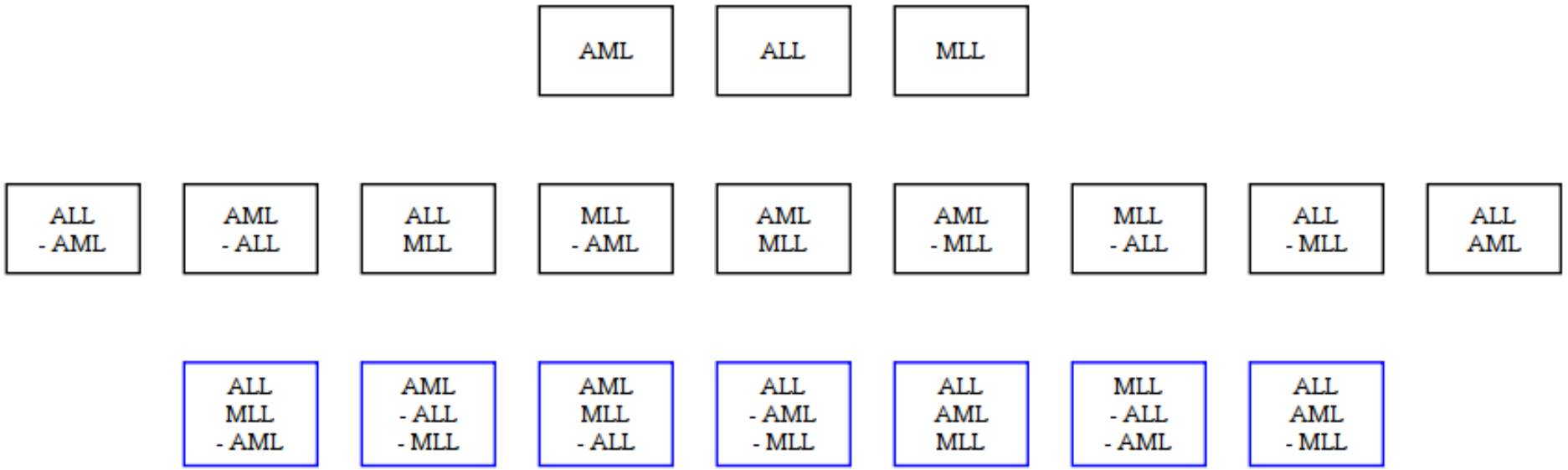
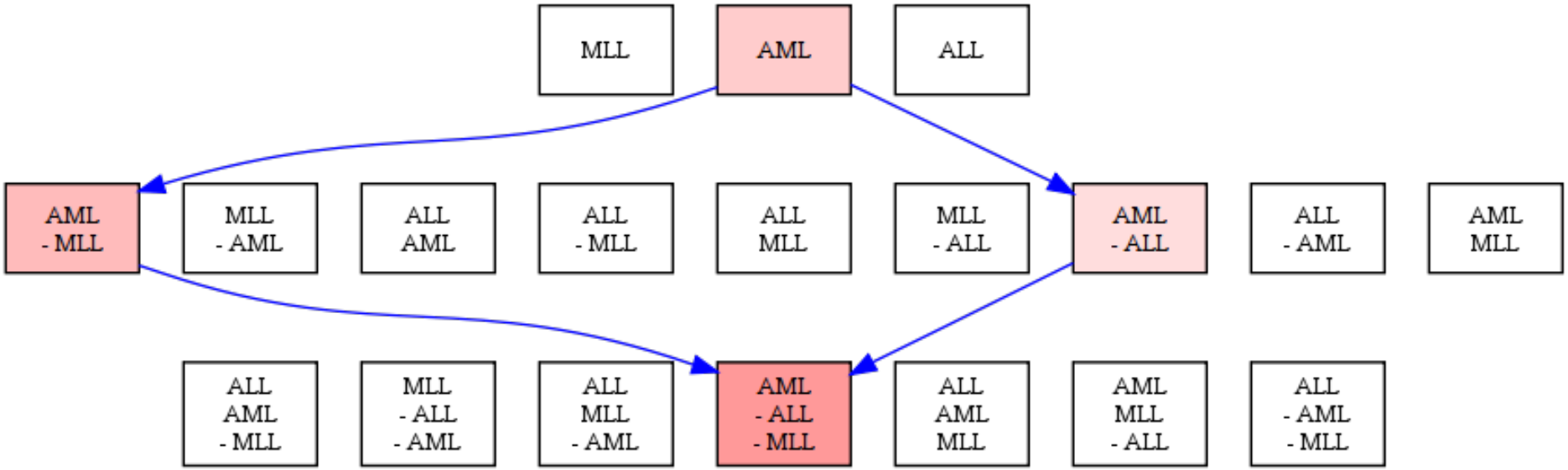


- Each node represents
  1. a boolean conjunction of (possibly negated) conditions and
  2. a network of interactions
- Can compute enrichment of known processes or pathways (Gene Ontology, Netpath, REACTOME, etc.) in each network.

Differential Activation of the Kit Receptor Pathway in AML



- AML: p-value 2×10^{-4}
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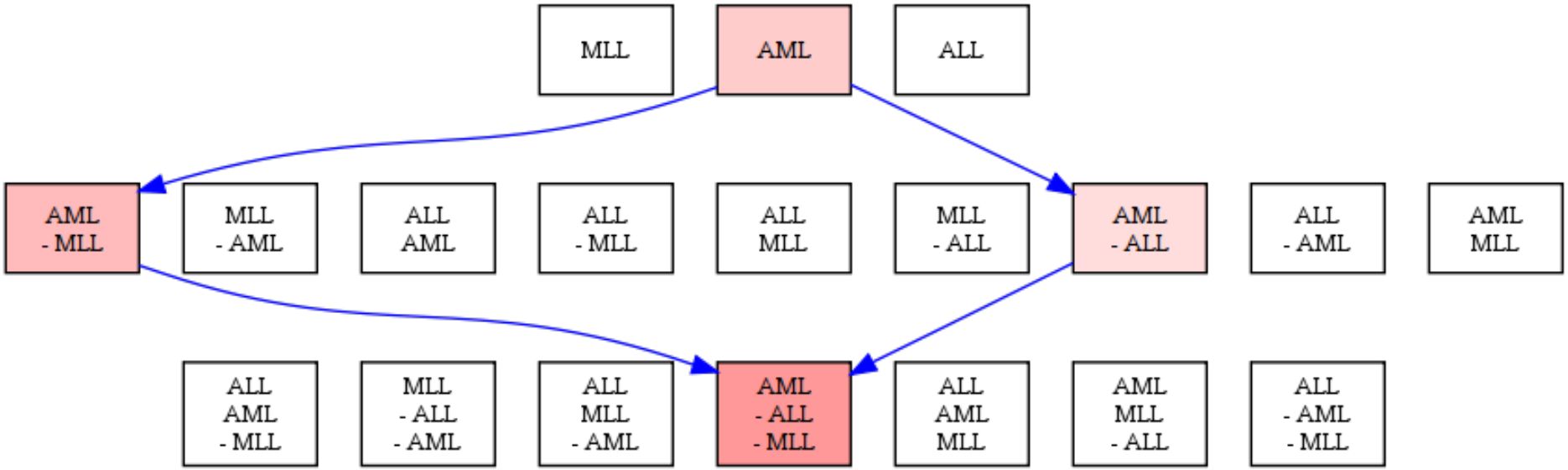

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- c-KIT receptor is activated in almost all subtypes of AML but not in ALL (Reuss-Borst et al., *Leukemia*, 1994, Bene et al., *Blood*, 1998, Schwartz et al., *Leuk Lymphoma.*, 1999).

Challenges in Comparing Arbitrary Numbers of Active Networks

- How can we efficiently compute all combinations?
- How do we identify which combinations are the network legos?
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  - Define and measure stability and recoverability.

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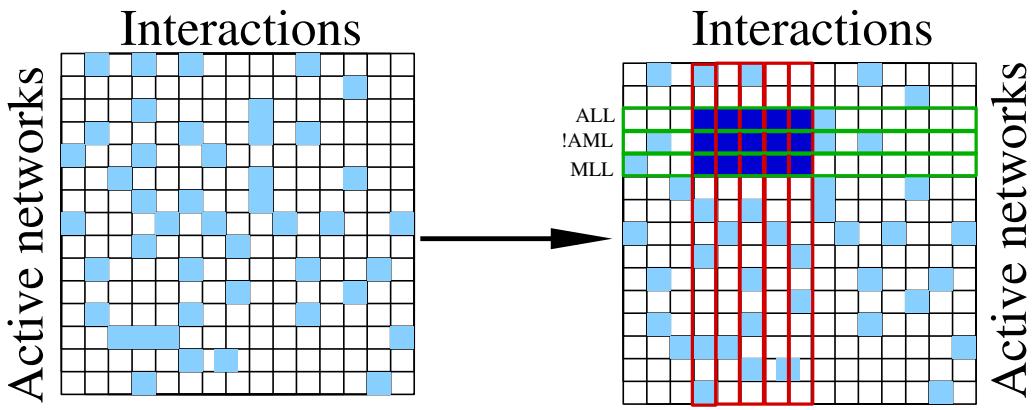
- Partial order exists between blocks, e.g.,
  - $\mathsf{ALL} < \mathsf{ALL} \cap \mathsf{AML}$.
  - $\mathsf{ALL} \cap \mathsf{MLL} < \mathsf{ALL} \cap \mathsf{MLL} \cap !\mathsf{AML}$.

Efficiently Compute Network Blocks

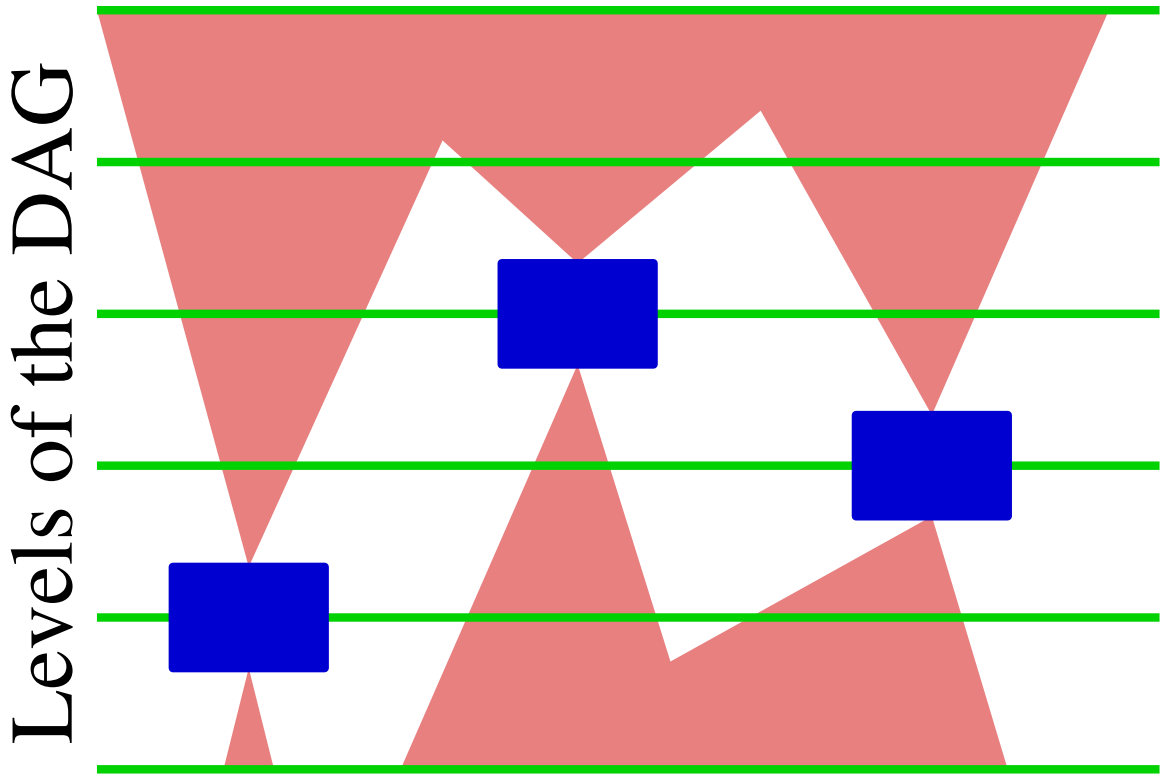


- Construct a binary matrix M whose columns are interactions.
- Represent each active network and its complement in M's rows.
- A *bicluster* is a subset of rows and subset of columns such that M only has 1s in this submatrix.
  - Rows of bicluster $\equiv$ formula.
  - Columns of bicluster $\equiv$ network.
- Compute all closed biclusters in M.
- Connect biclusters in the DAG induced by the partial order.

Assessing Statistical Significance of a Bicluster

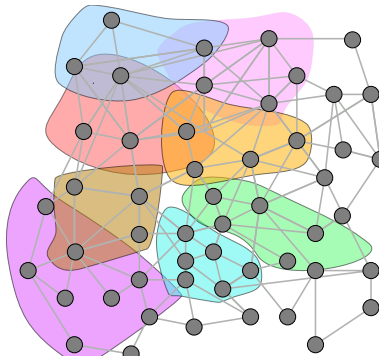
- Suppose a bicluster B has n included and c excluded active networks.
  1. Pick n active networks and the complements of c active networks repeatedly at random, compute the number of interactions induced by this combination, and build a distribution of the number of interactions.
  2. Set the p-value of B to be the fraction of random biclusters with more interactions than B.

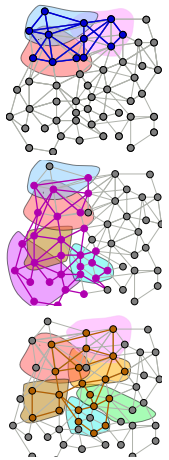
Identify Network Blocks that are Network Legos



- Assess the statistical significance of each bicluster by simulation.
- B is a *network lego* if it is more significant than any of its ancestors or descendants in the DAG.

Show that Network Legos are Building Blocks





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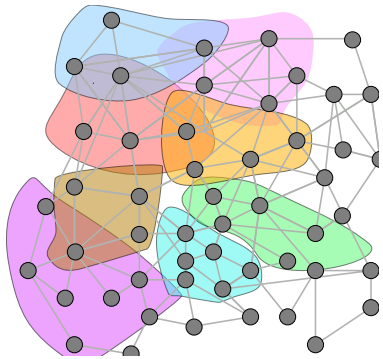

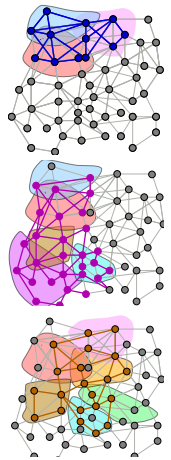

- Stability
  - Remove each active network and recompute network legos.
  - For each original network lego, compute the fraction of leave one out datasets for which the network lego occurs with at least t% fidelity.

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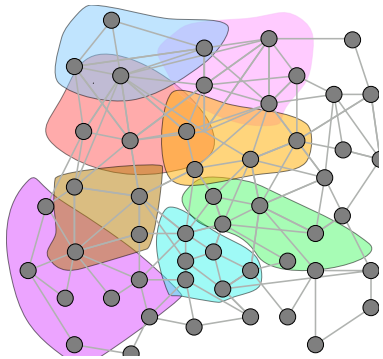

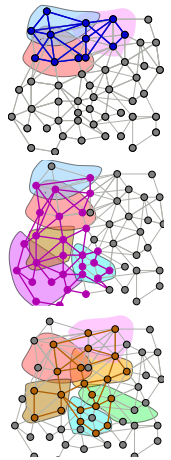

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- Recoverability
  - Compute the union of network legos.
  - Measure the size of the intersection of each active network with union.

Analysis of Human Stress Data

- Human protein-protein interaction network with 9243 proteins and 31000 interactions.
  - PPIs from (Ramani et al., Genome Biology, 2005; Rual et al., Nature, 2005; Stelzl et al., Cell, 2005).
- 13 distinct stresses applied to human cells (Murray et al., Mol. Bio. Cell, 2004).
  - Stress conditions include heat shock, oxidative stress, cell cycle arrest, and crowding.
  - Two cell types: WI38 Fibroblasts and Hela.
- Murray et al. note that each stress elucidated a unique response.

Human Stress Results

- 13 stresses and their active networks yielded 444201 closed biclusters.
- 143 biclusters are network legos.
- The network legos contained between 165 and 1148 proteins.
- Each network lego has 95% stability.
- The network legos provide better than 86% recoverability for all active networks.
- We recovered 11 active networks at 100%.

#conditions	5	6	7	8	9	10	11	12
#legos	1	6	10	36	34	20	28	8

Human Stress Results without Cell Cycle Arrest Treatment

- The active networks for cell cycle arrest treatments contain interactions that are distinct compared to those in active networks for other treatments.
- 11 stresses yielded only 15 network legos.
- The network legos provide better than 71% recoverability for all active networks.
- We recovered five active networks at 100%.
- Each formula contained at least 7 active networks.

WI38 Response to Menadione and DTT

- One network lego contained endoplasmic reticulum stress and oxidative stress to fibroblasts in non-complemented form.
- All other stresses appeared in complemented form.
- This network lego is the only one enriched in functions related to the cell cycle and targets of the E2F1 transcription factor.
- Fibroblasts respond differently from HeLa cells to these two stresses.

Our Contributions

- Combined representation of biological processes using formulae and network legos.
- A formula relates different cellular states or perturbations by explicitly denoting their participation via intersections and complements.
- Each network lego corresponds to a functional module of coherently interacting genes in the wiring diagram.
- Network legos serve as building blocks of active networks.

Compendium Approach