Genome-wide discovery of modulators of transcriptional interactions in human B lymphocytes

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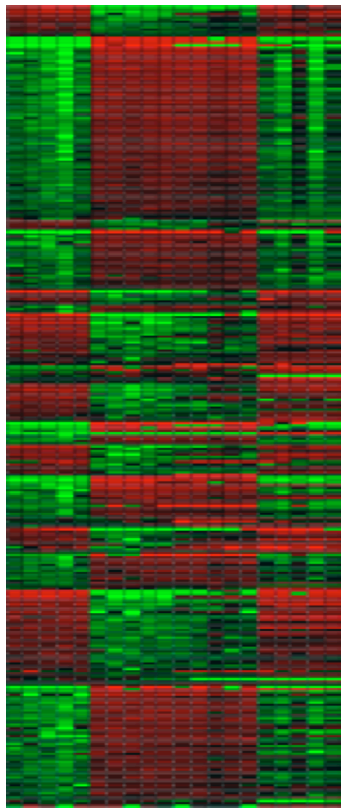
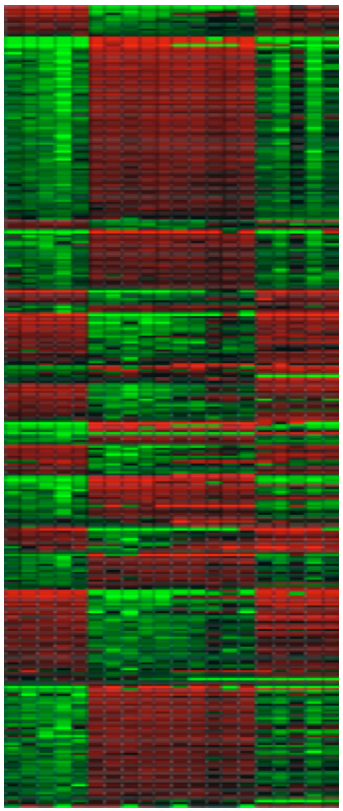
Reconstructing cellular interactions

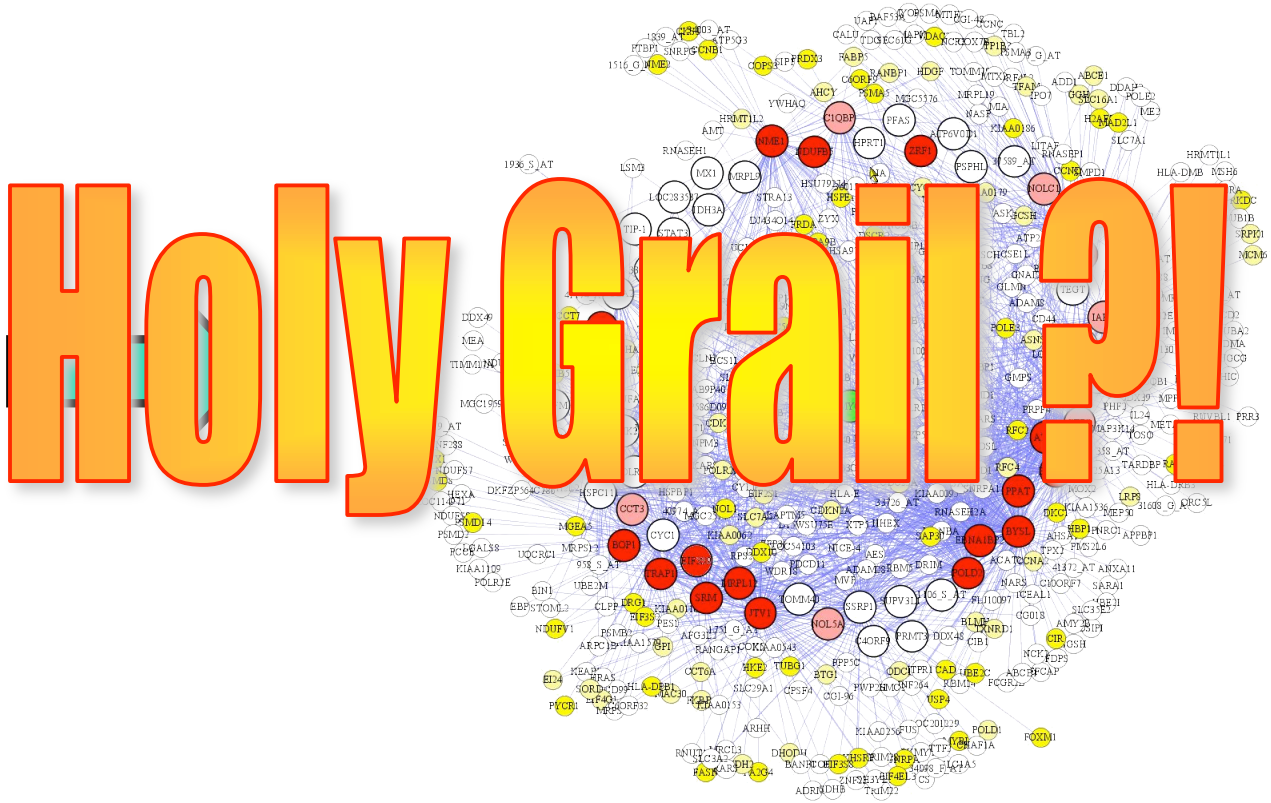


HG-U95A (approximately 12,600 probes)

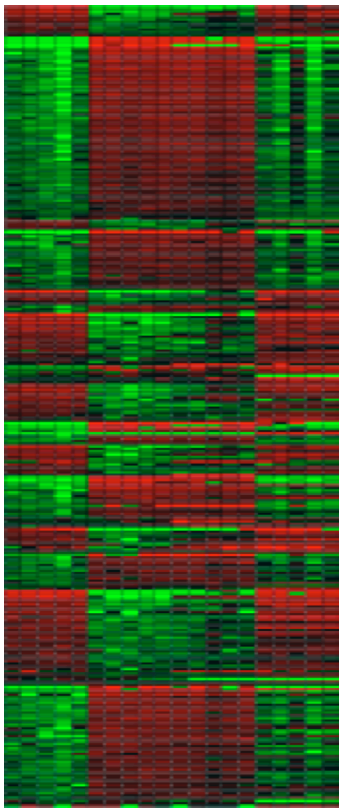
- Over-, under- expression, diagnostics tools (since late 1990s)
- Clustering, pathways identification (since late 1990s)
- Interaction networks (since early 2000s)

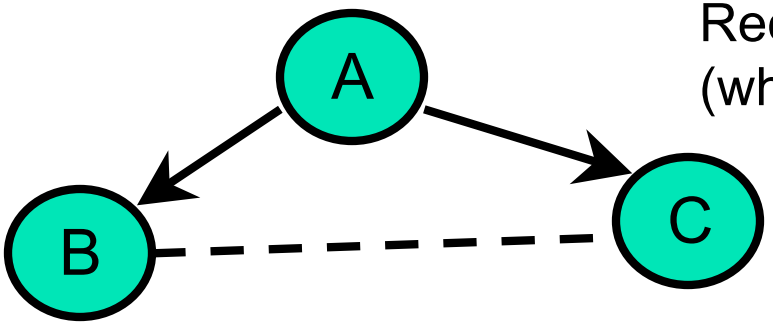
Reconstructing cellular interactions



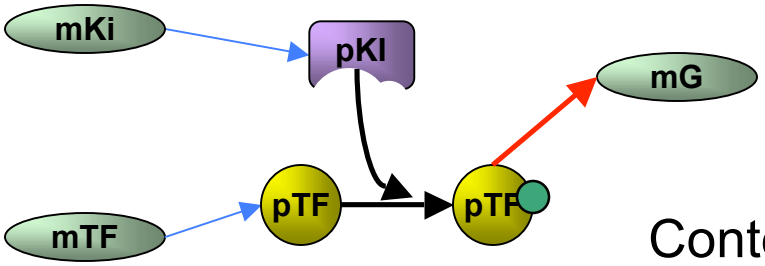


Two major problems



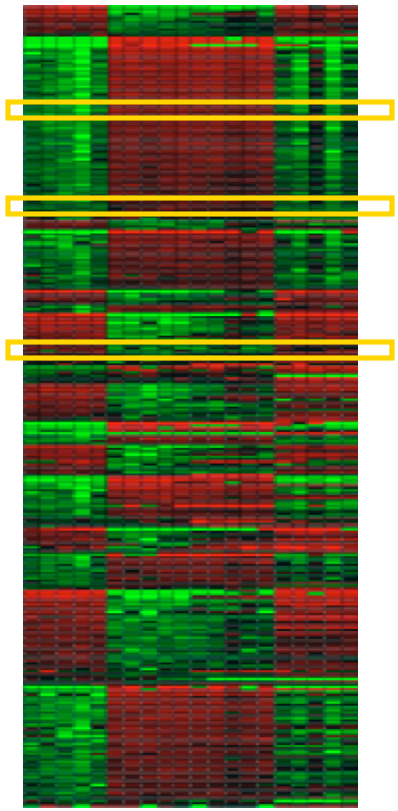


Reducibility
(what is an interaction?)



Context specificity
Posttranslational modifications

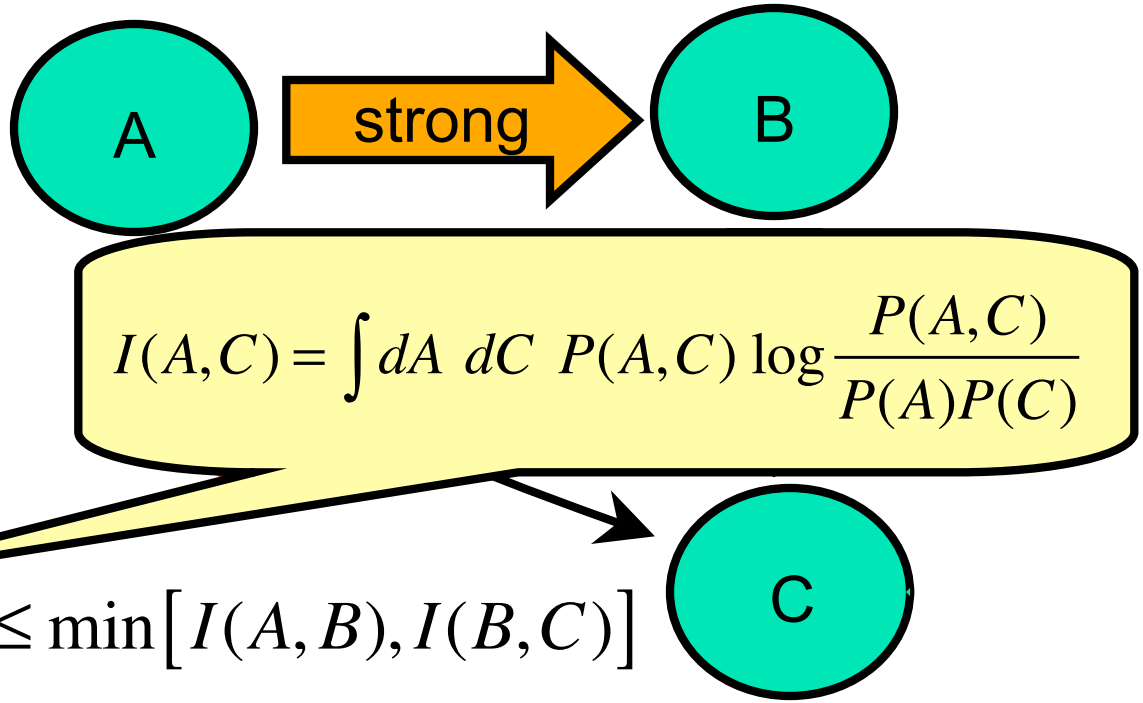
Problem 1: ARACNE
(Data Processing Inequality, DPI)



A

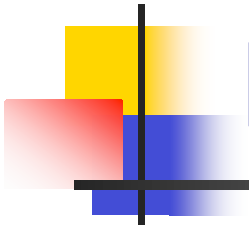
B

C



Reparm. invariance; small sample; low complexity.

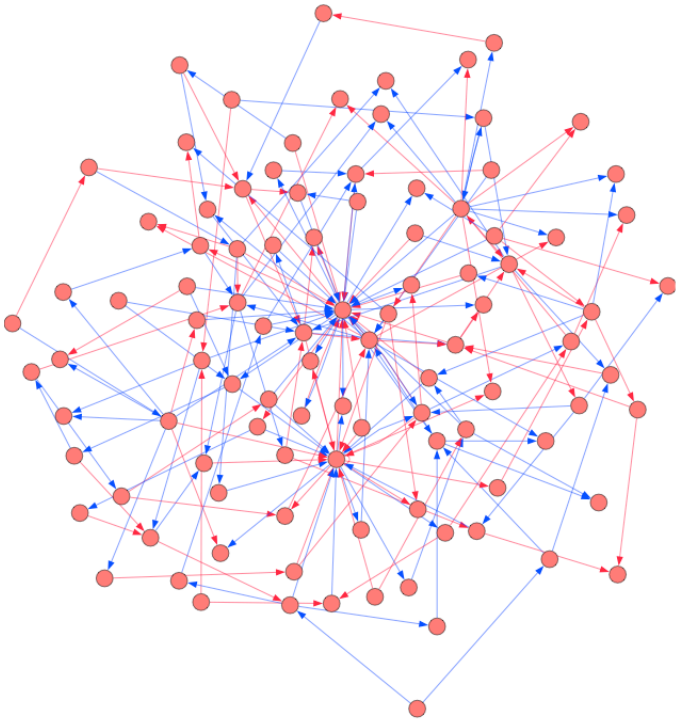
Performance?

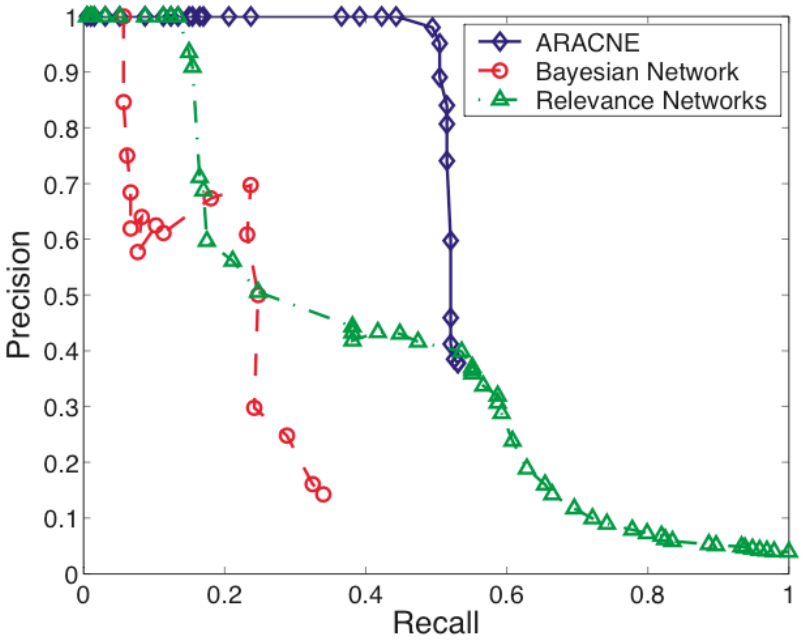


Performance: Few false positives

- No false positives for tree networks
- No false positives under very general conditions for networks with few strong loops
- No false negatives under stronger conditions
- Need to estimate MI reliably

Synthetic networks

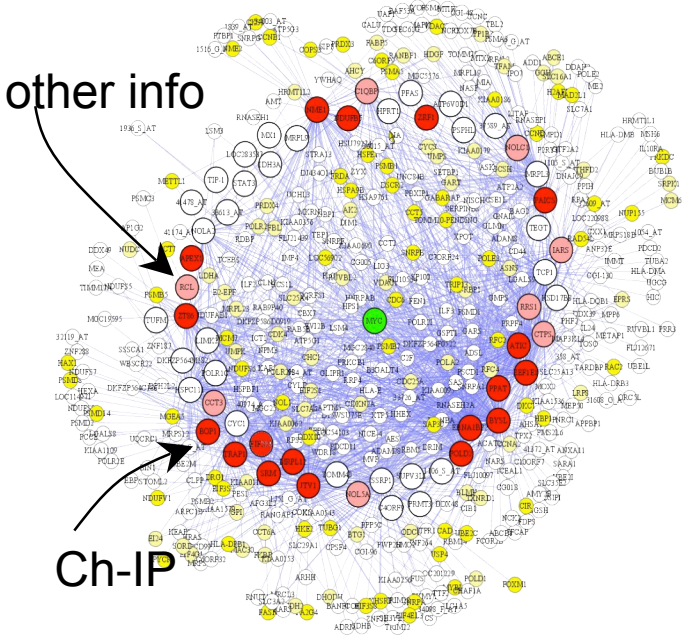




$$\frac{dx_i}{dt} = a_i \prod_j \frac{I_{0,j}^{\nu_j}}{I_j^{\nu_j} + I_{0,j}^{\nu_j}} \prod_j \left(1 + \frac{A_{0,j}^{\nu_j}}{A_j^{\nu_j} + A_{0,j}^{\nu_j}} \right) - b_i x_i$$

B-cell dataset: cMYC network

- ~400 arrays (Dalla-Favera et al.)
- No dynamics
- ~250 naturally occurring, ~150 perturbed
- ~25 phenotypes (normal, tumors, experimental perturbations)



- Protooncogene,
- 12% background binding,
- one of top 5% hubs
- significant MI with 2000 genes

Total interactions: 56
Pre-known: 22
New Ch-IP validated: 11/12

Problem 2: posttranslational modulation from mRNA data

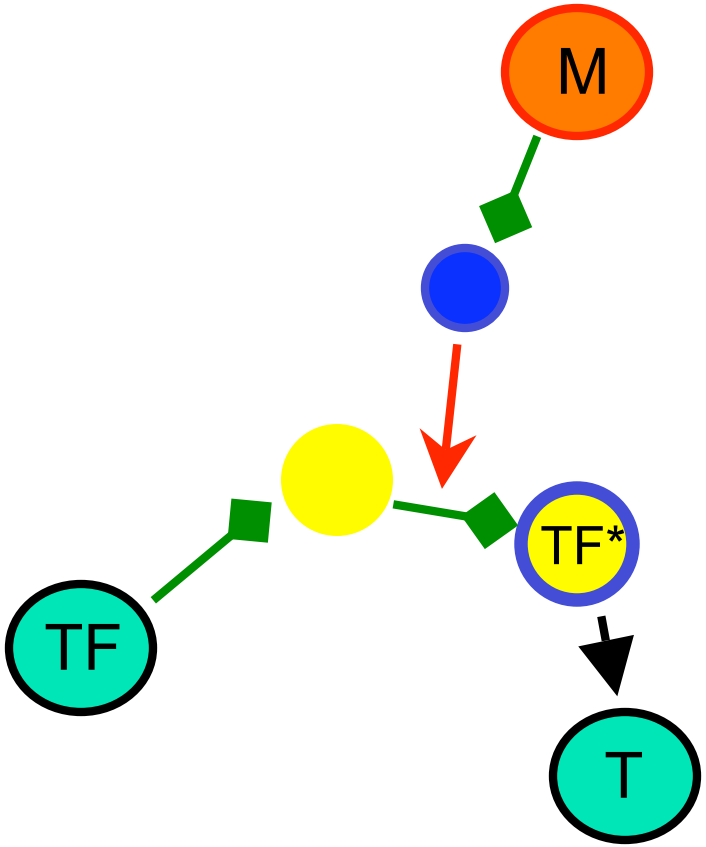
- Much of regulation in higher eukaryotes is post-transcriptional (e.g., splicing), and post-translational (e.g., phosphorylation, complex formation).
- Many mRNA (e.g., p53) constitutively expressed.

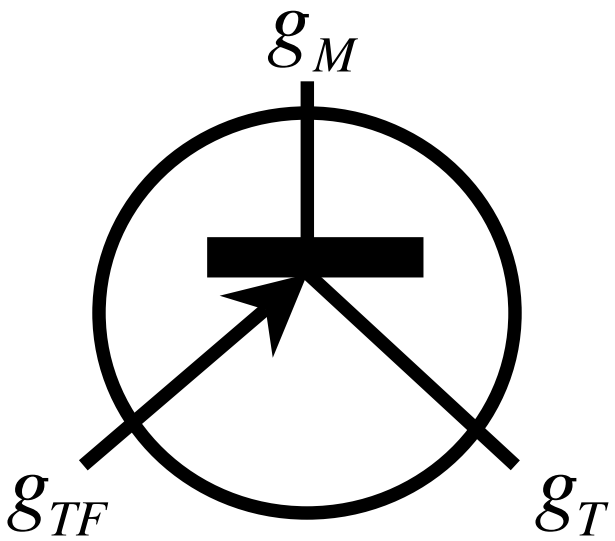
Can these be observed from mRNA expressions only?

Solution:

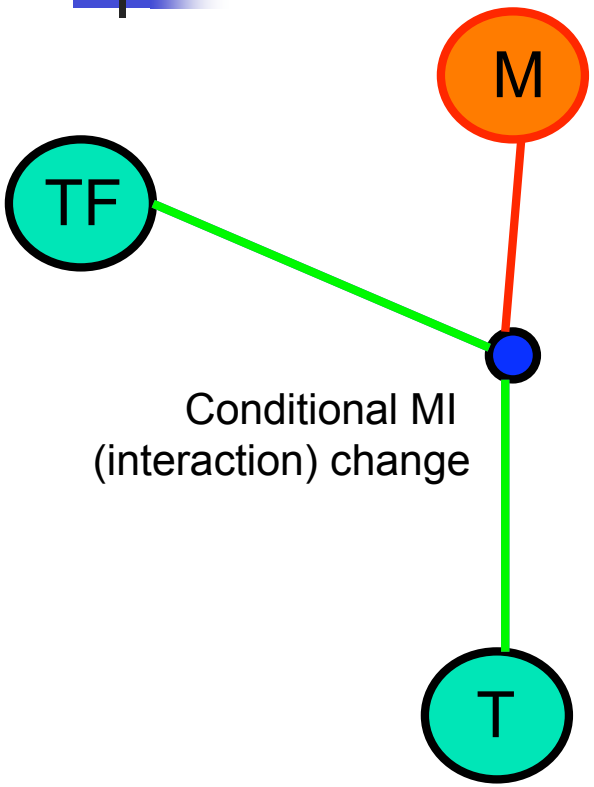
Phenotypic and population variability (even in constitutively expressed genes) induces higher order dependencies between TFs, targets, and modulators.

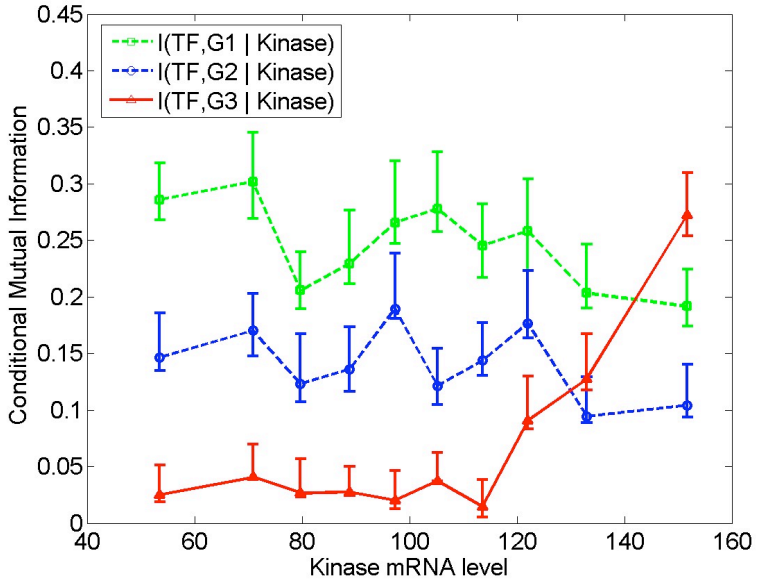
Posttranslational modulation: a transistor model

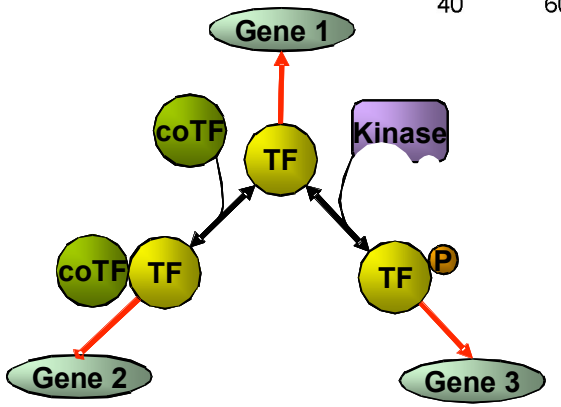




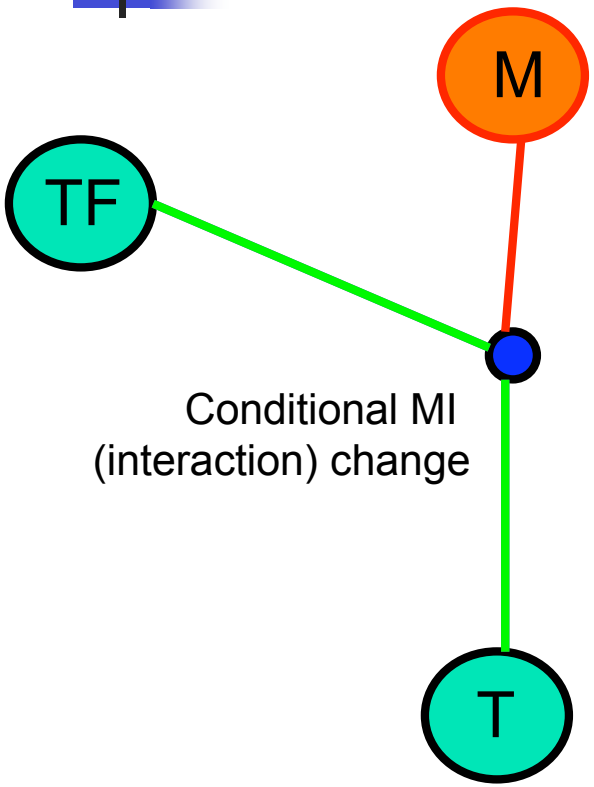
Posttranslational modulation: MI signature

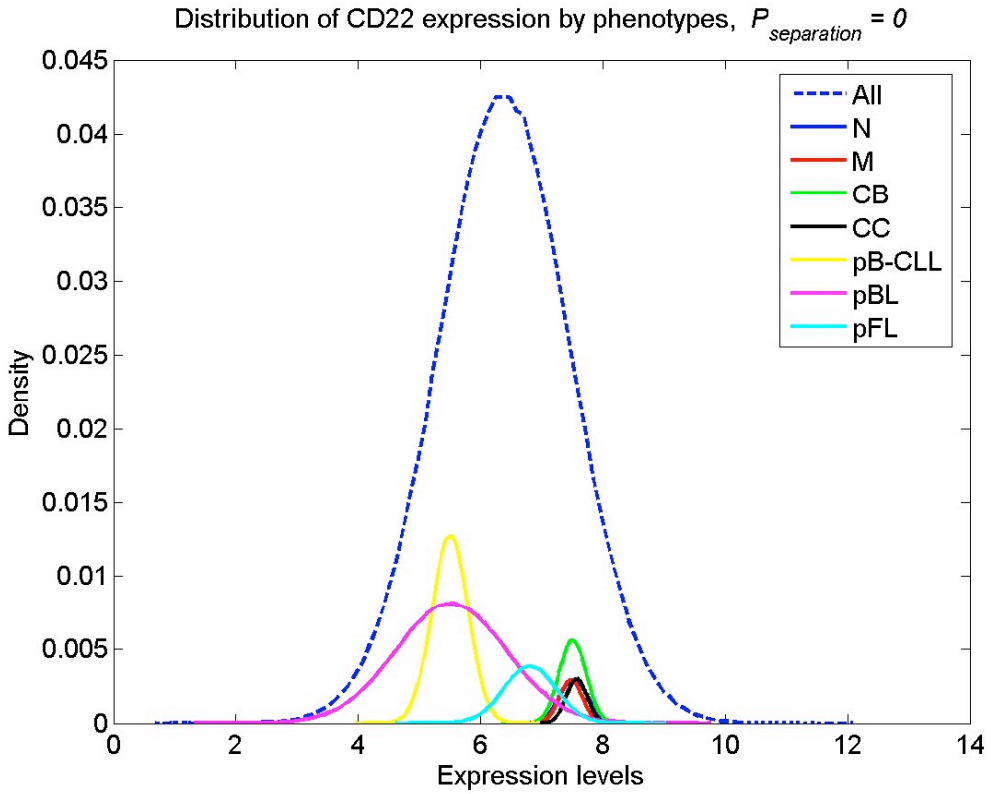




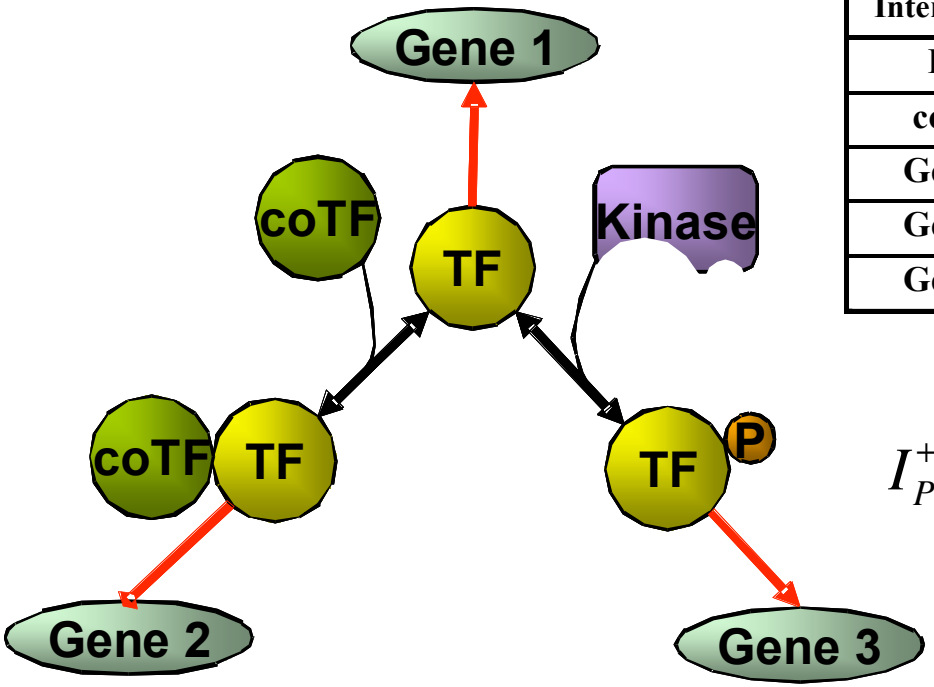


Phenotypic variability of constitutive modulators





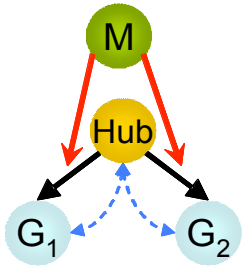
Numerical case study: Transistor modulation

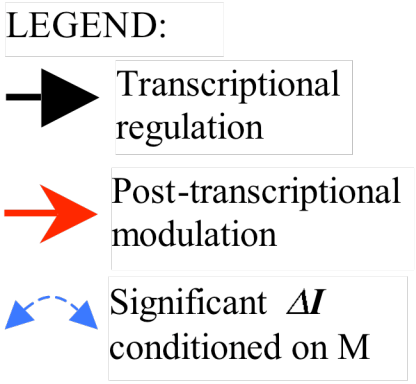


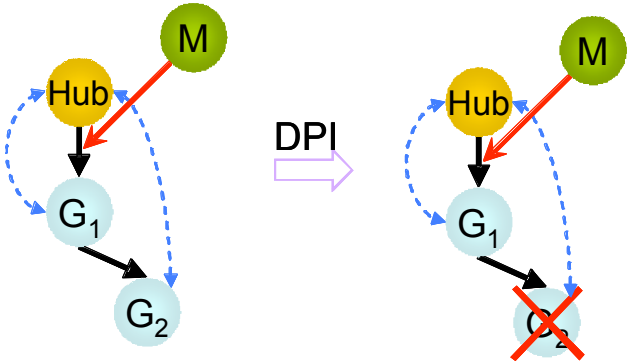
TF Interaction	I	I^{-}_{PK}	I^{+}_{PK}	ΔI_{PK}	I^{-}_{coTF}	I^{+}_{coTF}	ΔI_{coTF}
PK							
coTF							
Gene1	0.73	0.54	0.57		0.55	0.54	
Gene2						0.37	0.37
Gene3			0.35	0.34			

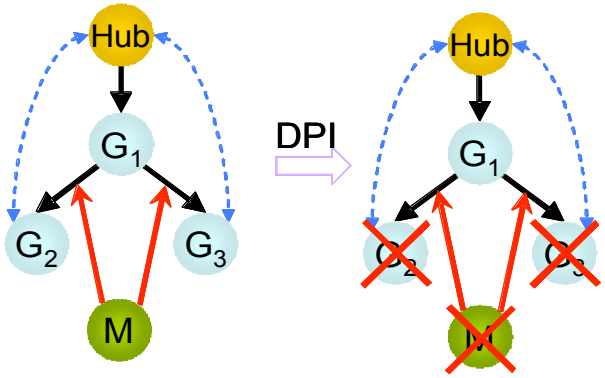
$$I^{+}_{PK}(\text{gene1}) = I(TF, \text{gene1} \mid PK \text{ high})$$

Enforcing irreducibility: ARACNE on a TF-hub

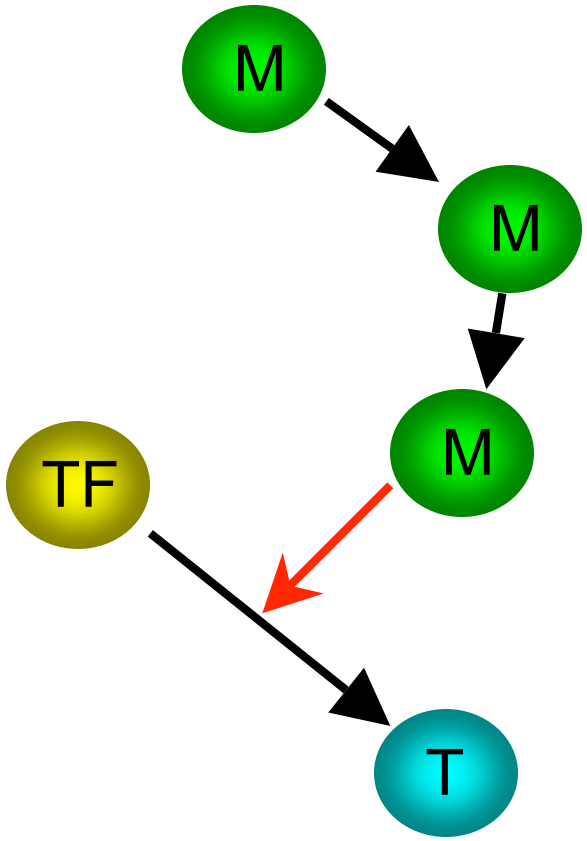








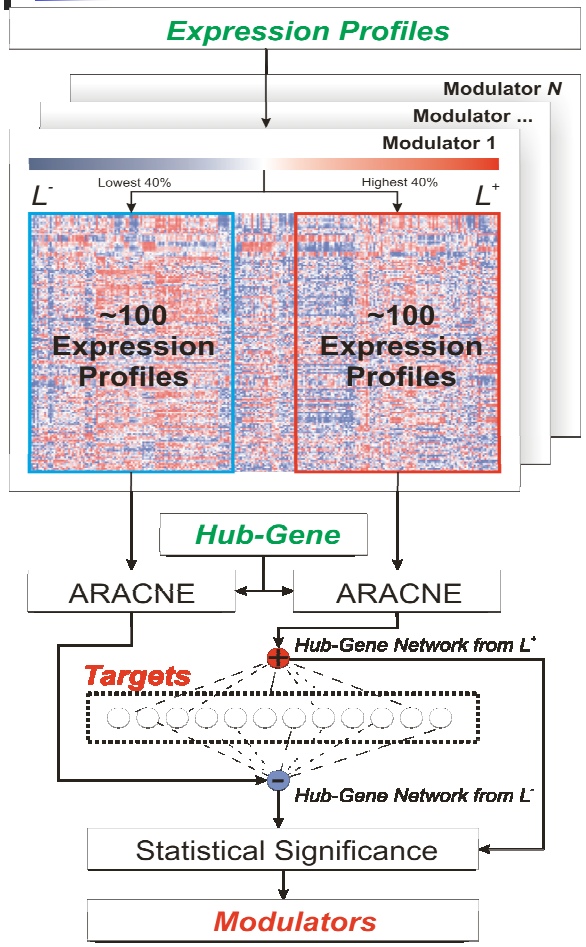
However:
No solution yet for…



Modulators are not irreducible.

Any suggestions?

Algorithm flowchart



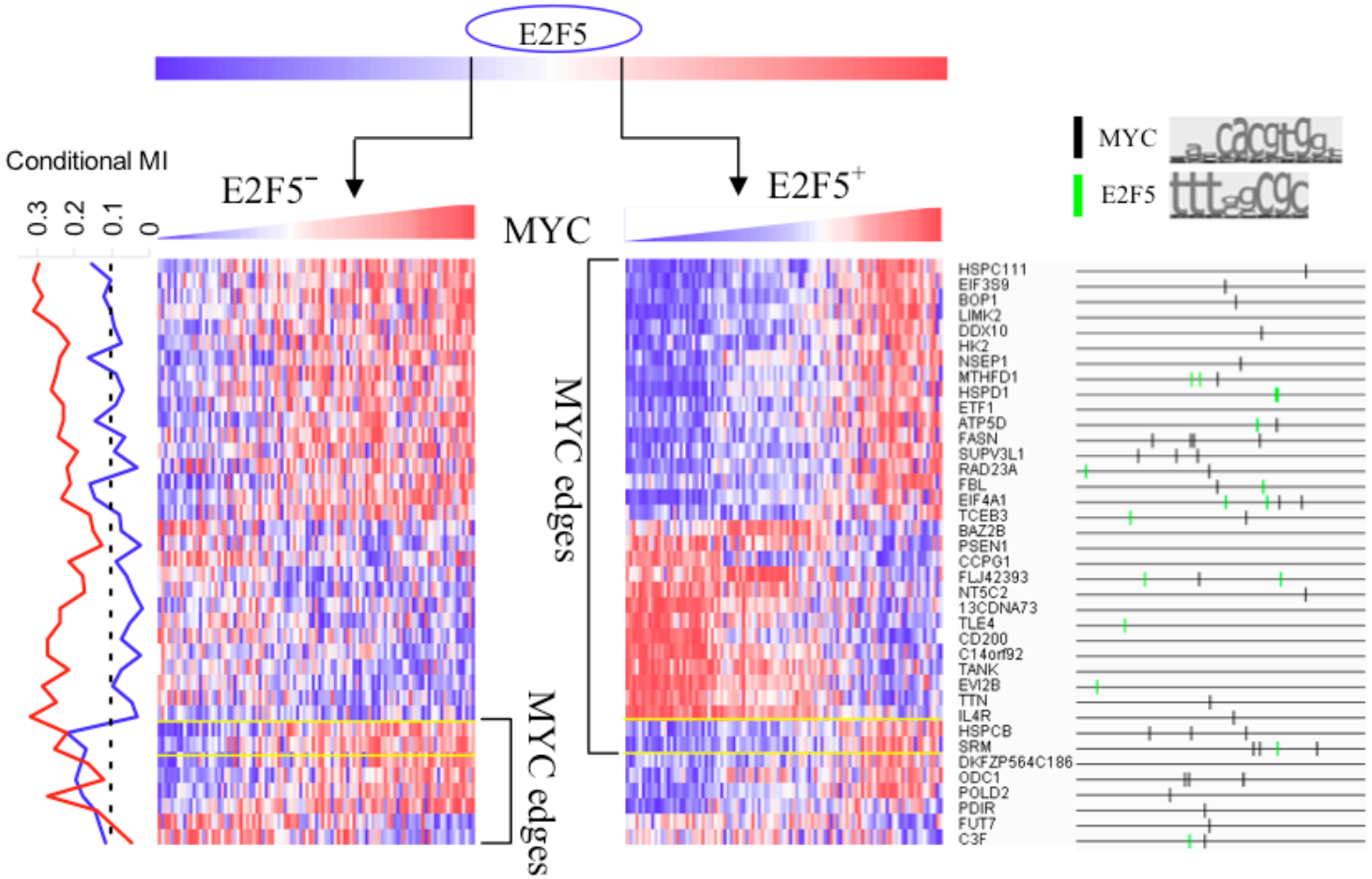
- Focus on a hub (c-MYC).
- Select modulators with σ> microarray noise (Tu et al., 2002) -- many signaling genes, constitutively expressed genes.
- Find modulators whose expression inflicts significant conditional MI changes for an ARACNE target in at least one conditional topology.
- No guarantee of modulator irreducibility.
- Guarantee of target irreducibility (after multiple hypothesis correction).

$$\Delta I(g_{TF}, g_t \mid g_m) =$$
$$= \left| I(g_{TF}, g_t \mid g_m^+) - I(g_{TF}, g_t \mid g_m^-) \right| > 0$$

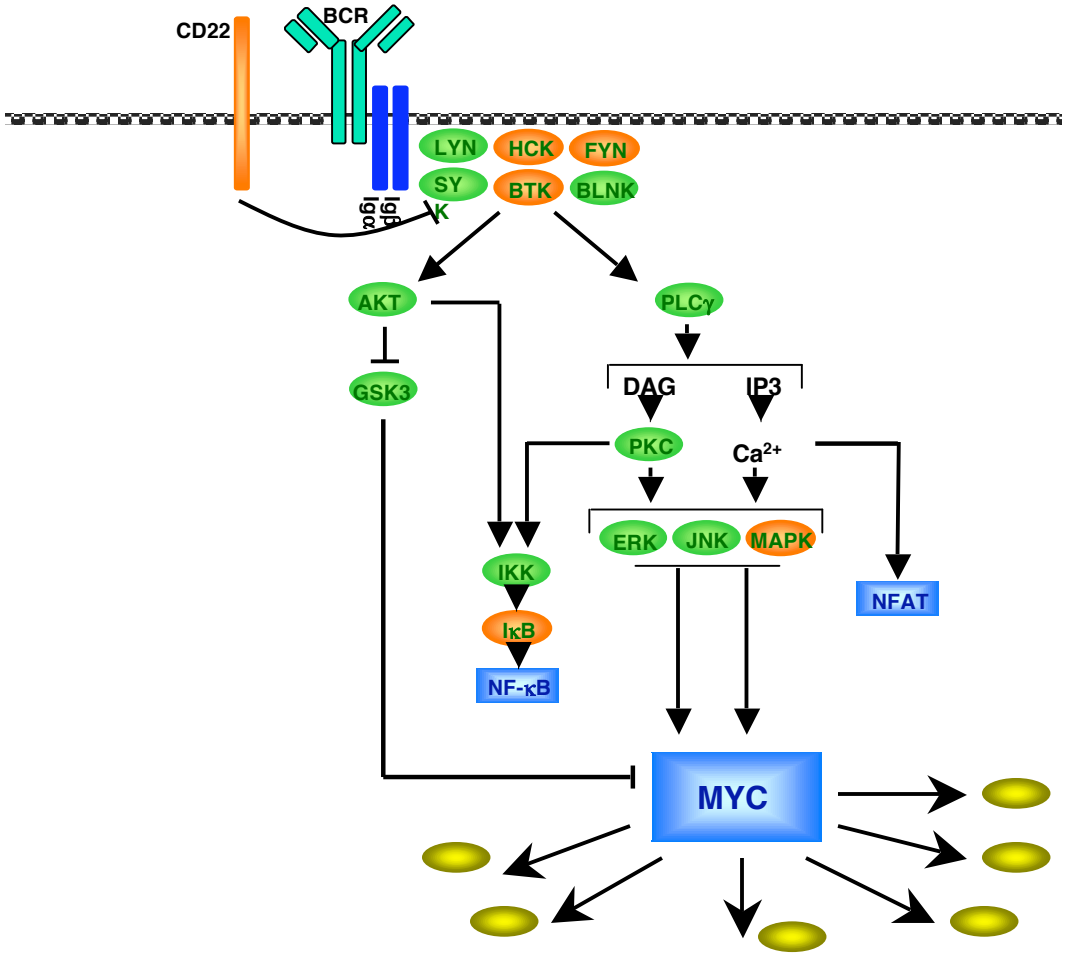
c-MYC modulators

- 1117 candidate modulators
- 100 modulators, 130 targets, 205 interactions
- GO enrichment of the modulator set: kinases, acyltransferases, TFs (all p<5%)
- Modulators in known MYC regulation pathways (e.g., BCR)
- TFs: 15/100, p=1e-6.
- 4/5 TF modulators (e.g., E2F5) with TRANSFAC signatures have binding sites in modulated targets promoter regions.
- Modulators with many (>=4) targets are not-specific (proteolisis, upstream signaling components, receptor signaling molecules).
- Modulators with few (1-2) effected targets are mostly co-TFs, interaction-specific.
- ~1/3 modulators are literature-validated.
- Biochemical validation of predictions in progress.

Example:
TF co-factor modulator



BCR pathway: Reducibility



predicted modulators

not in the candidate list

TF's not predicted

Protein complex

Targets