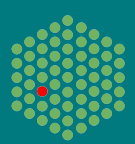# Integration of functional genomics & pathway information to elucidate deregulation of signal transduction and drugs' mode of action
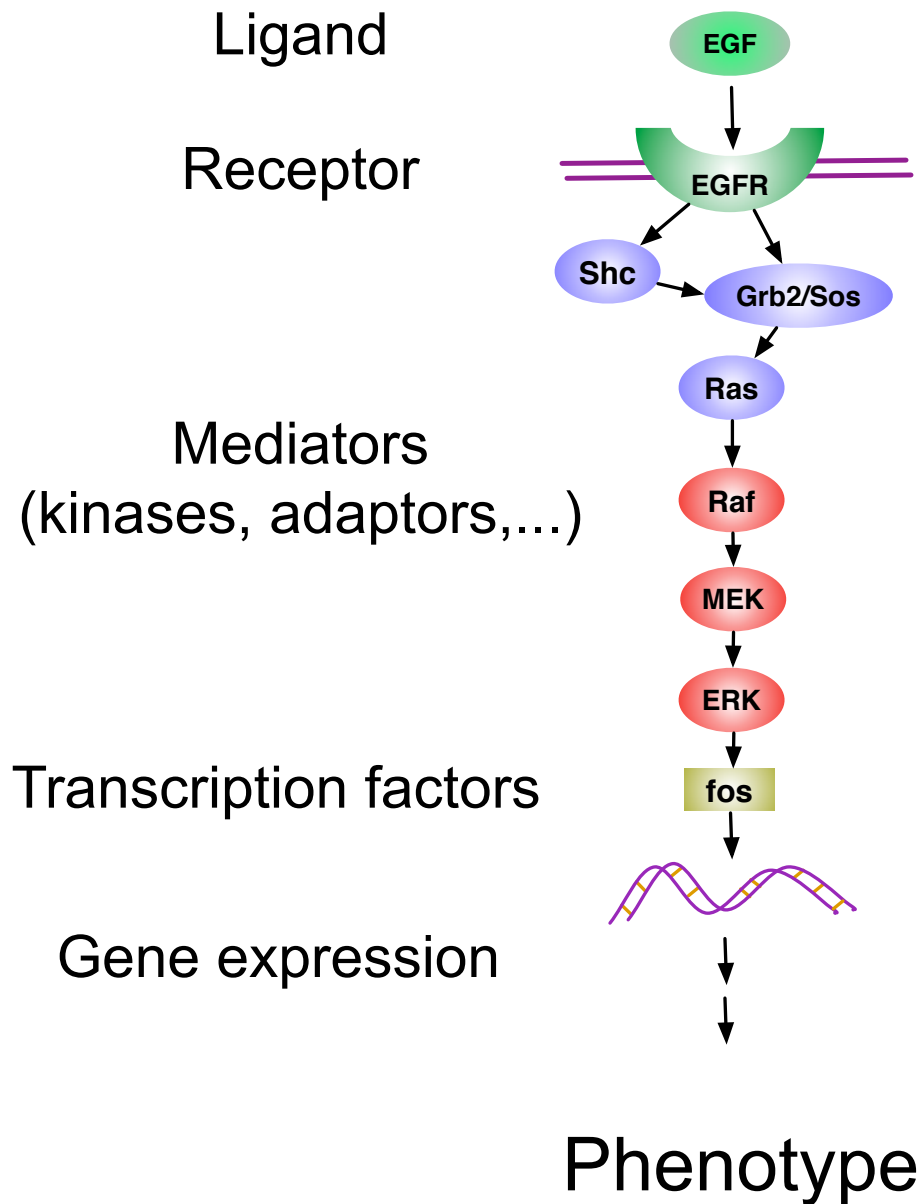
**Michael Schubert
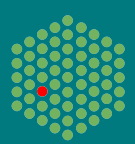& Julio Saez-Rodriguez**

**European Bioinformatics Institute
Hinxton (Cambridge) UK**

www.ebi.ac.uk/saezrodriguez

How do cells process extracellular signals?
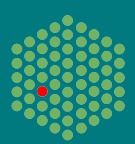

Phenotype
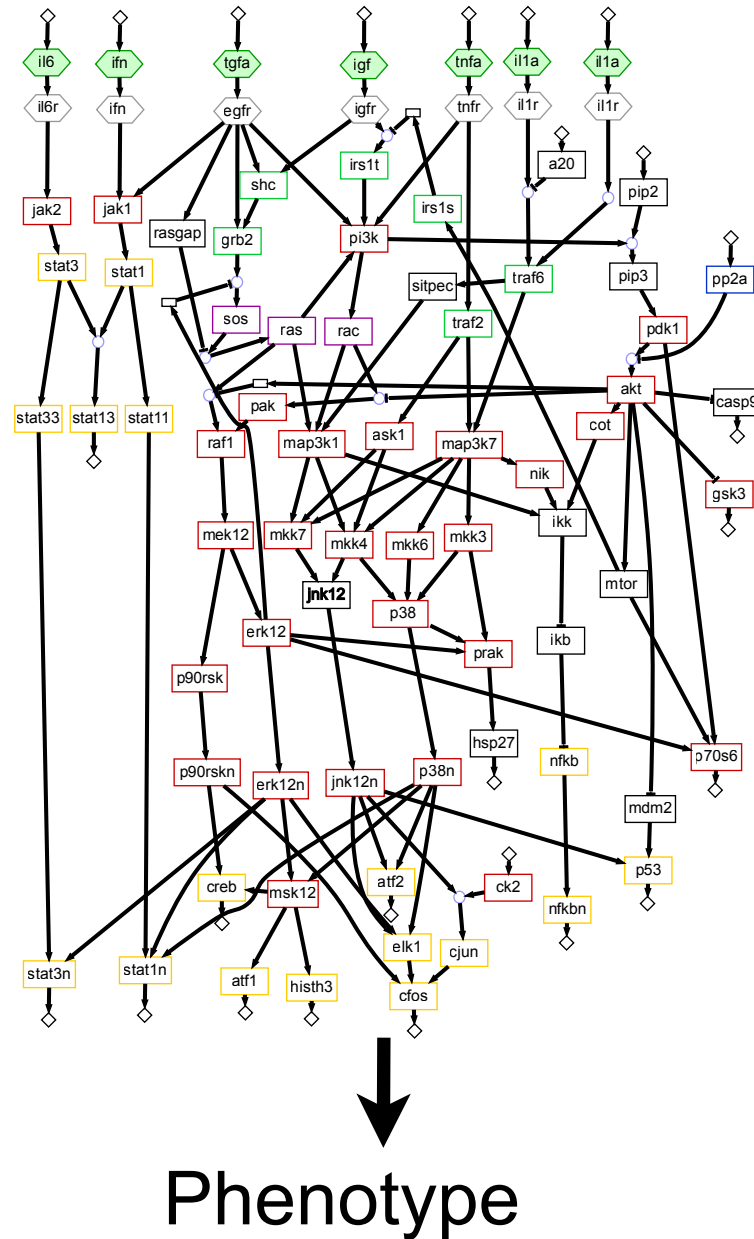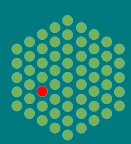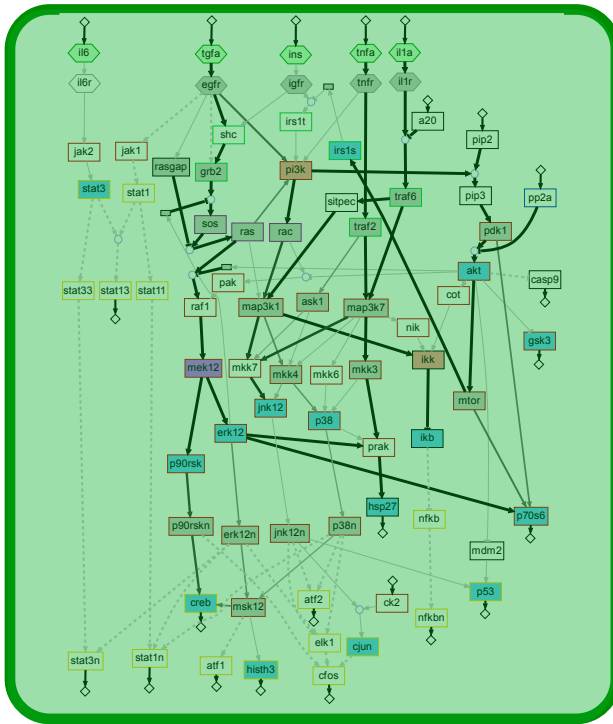
Phenotype

How is signal processing altered in disease?

Normal

Diseased
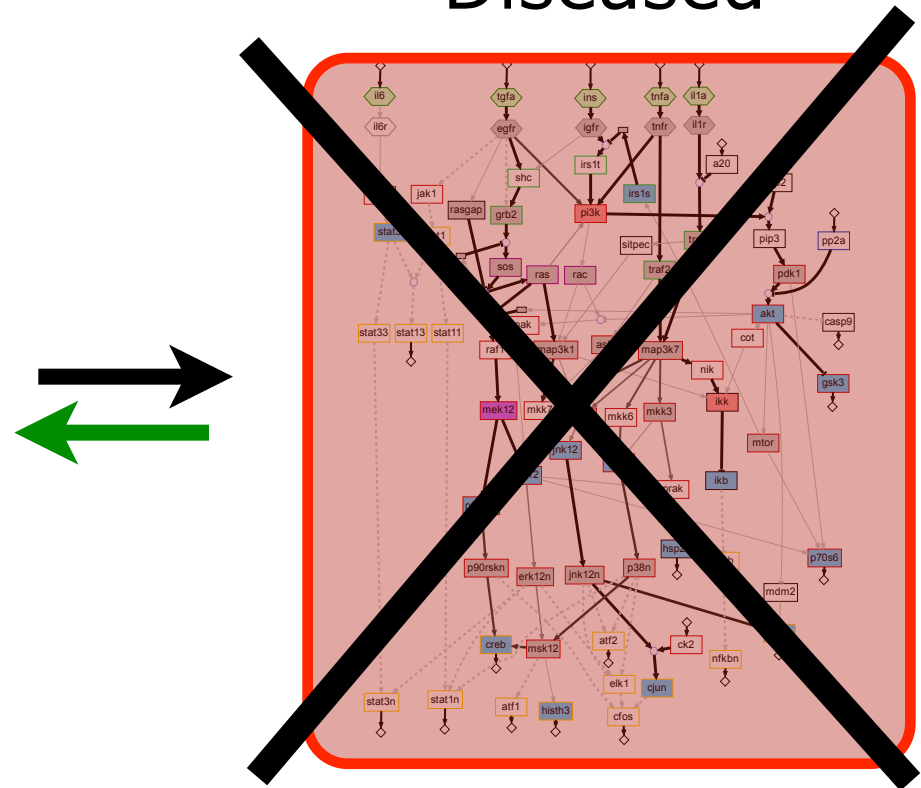
Can we revert disease phenotype ...
or target diseased cells with new therapies?

- Identify drug targets for a certain disease

- Characterization of mode of action

protein+drug complex

Therapeutic Target (known)

Off Target

Drug molecule

?
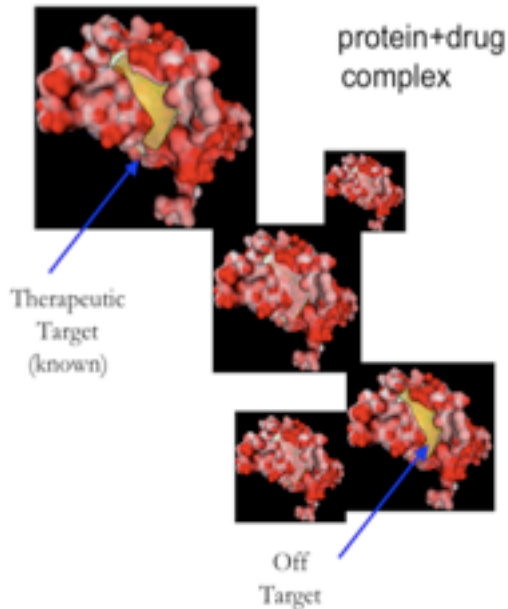
Identifying the molecular pathways targeted by a compound and its off-target effects

Dissecting what follows functionally the drug/substrate interaction

Gene Regulation

DNA

New Proteins

Response to Stress

Signals to other Cells

Homeostatic Regulation

Metabolism

?
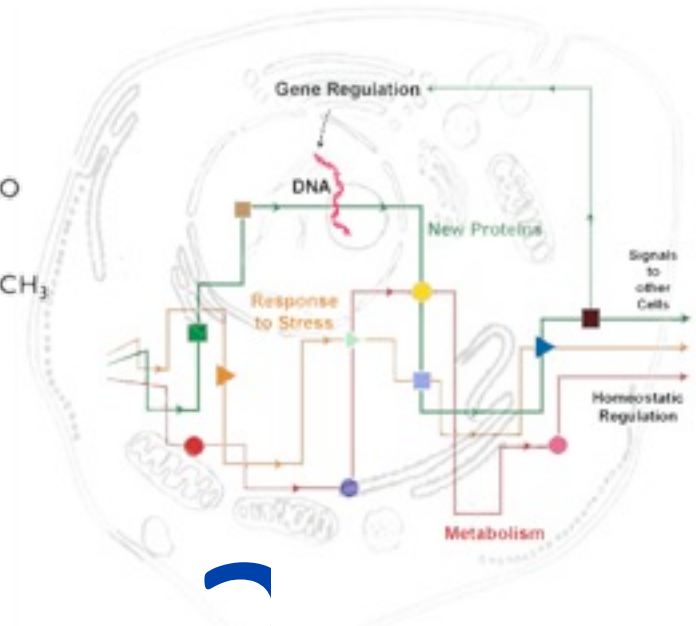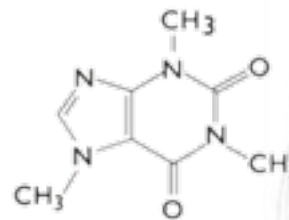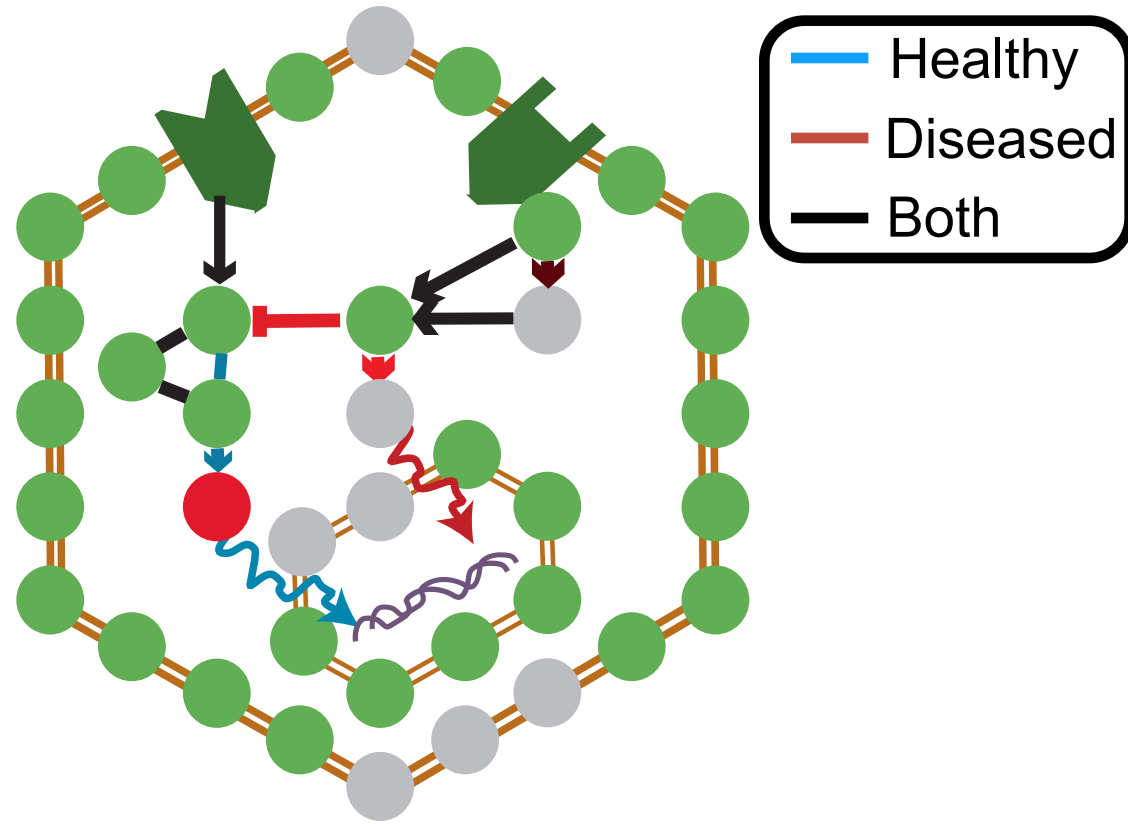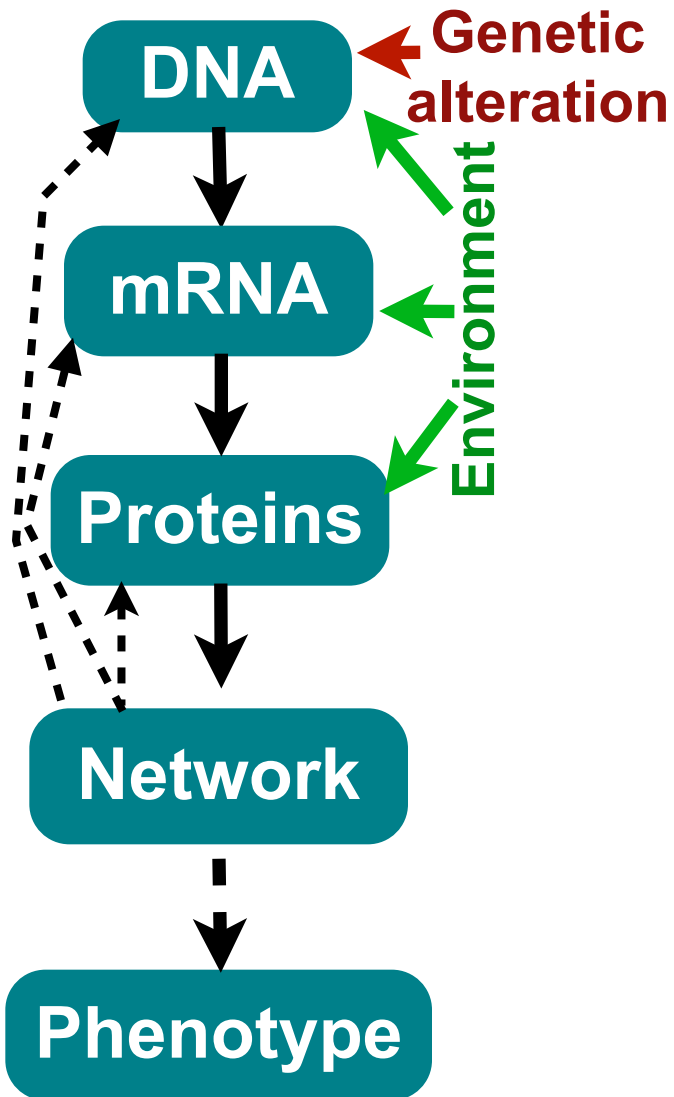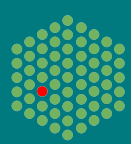
Characterization of drugs at biochemical level with mechanistic models of signalling networks

DNA

mRNA

Proteins

Network

Phenotype

Perturb with drugs and ligands

Measure phosphorylation of proteins
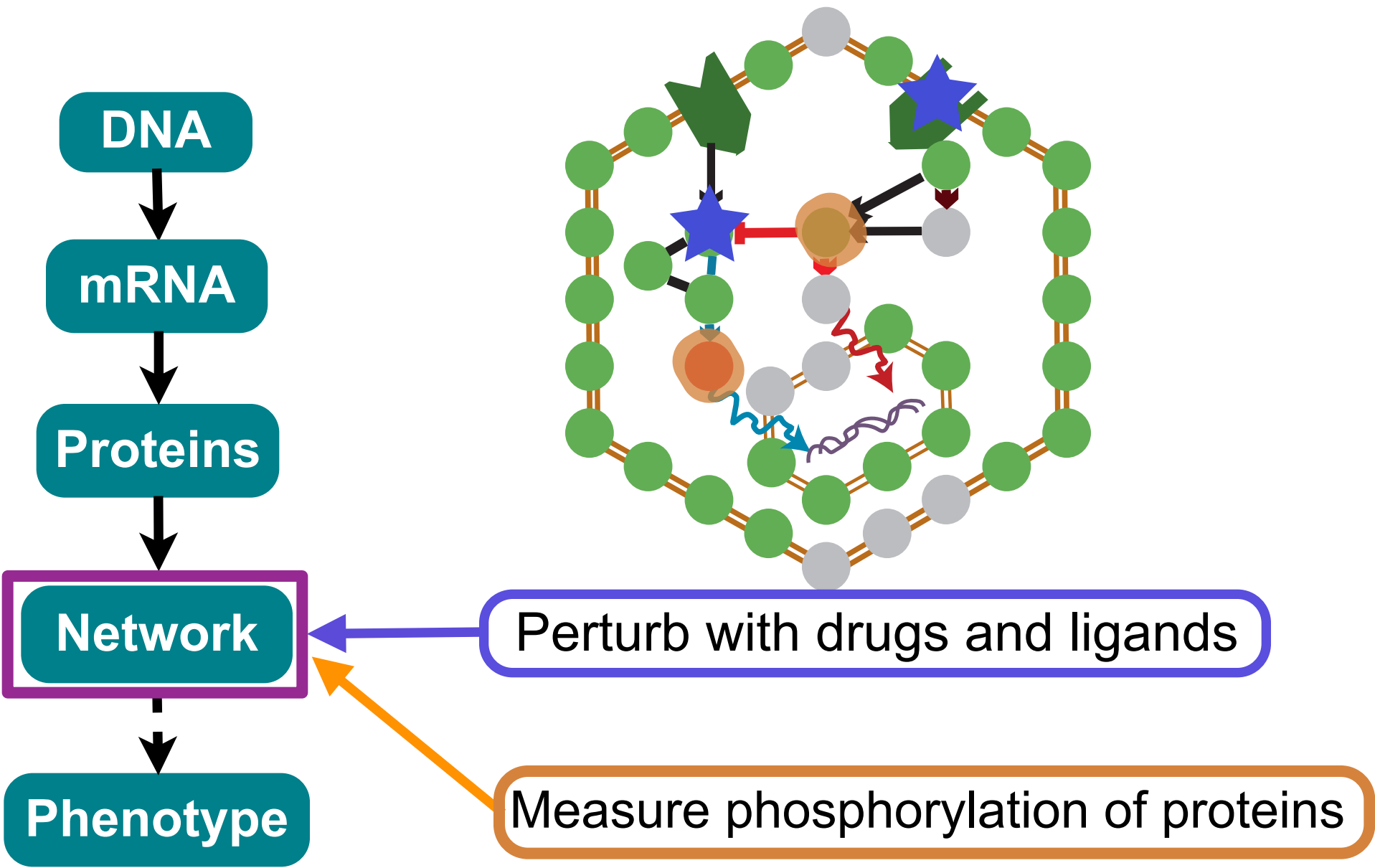
Comparison of primary hepatocytes and hepatocelluar carcinoma using logic models
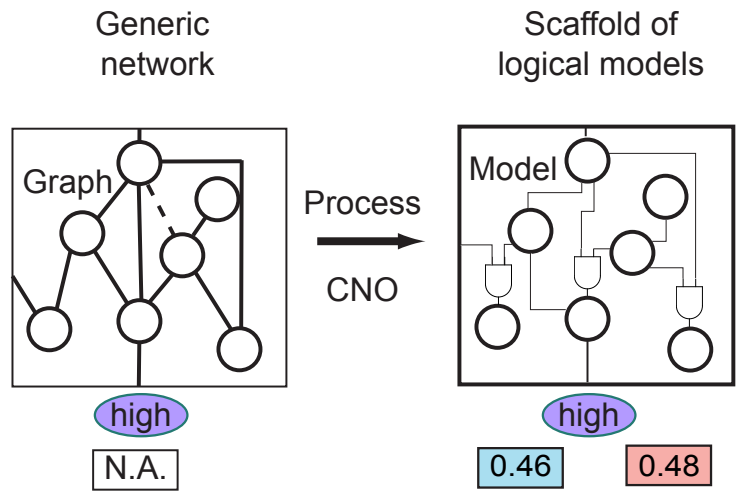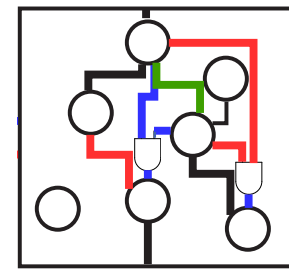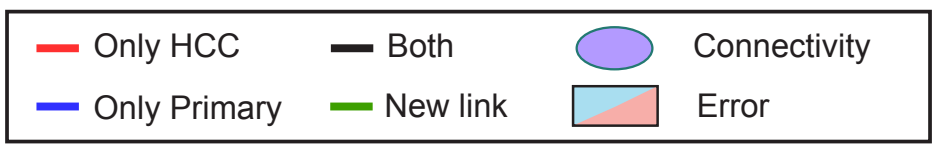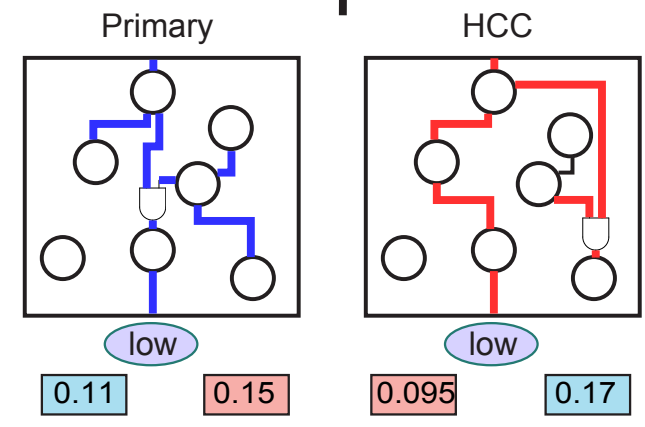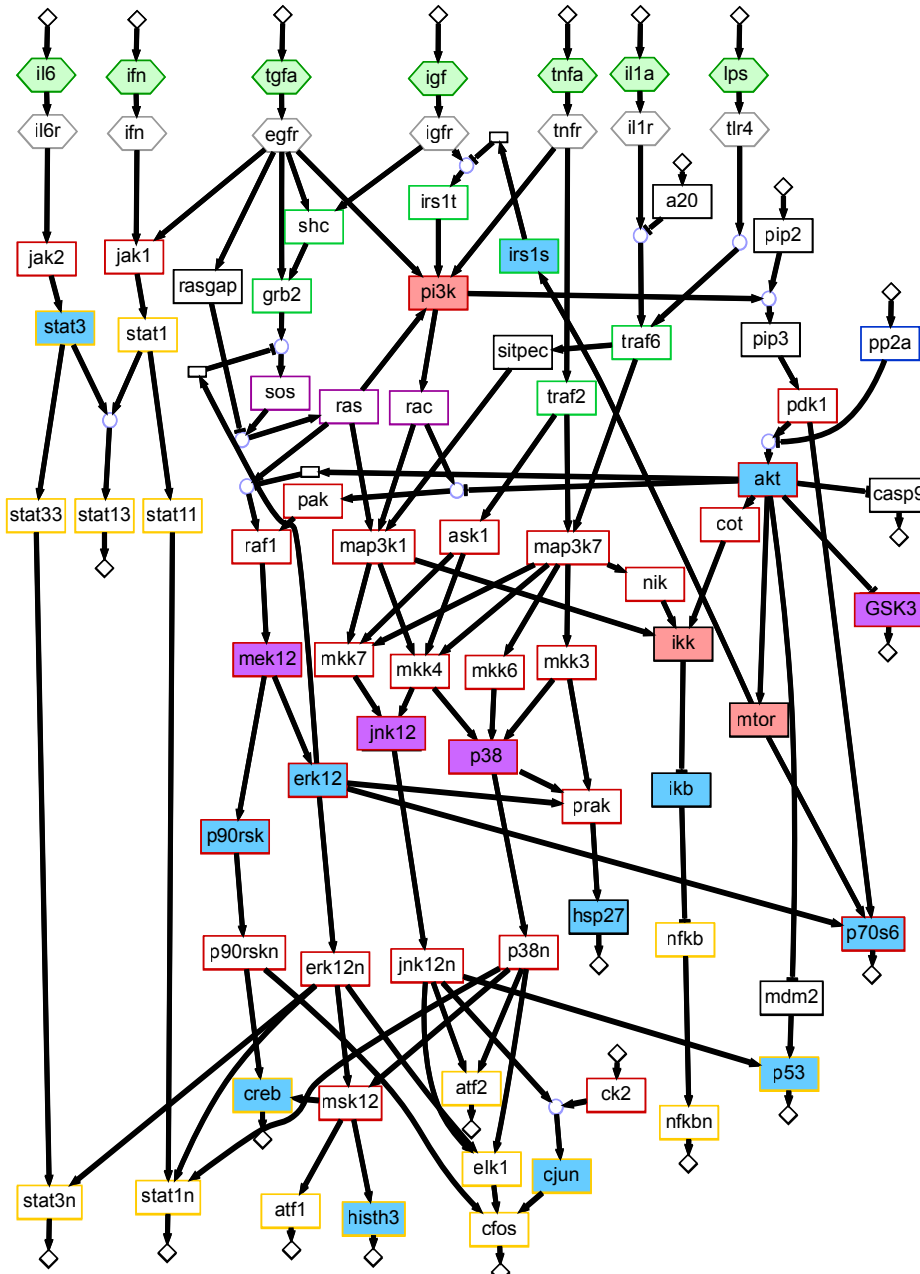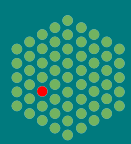
Construct map of canonical pathways

Select

- **perturbations** (chemical inhibitors = drugs) &
- **signals** (phosphorylations measurable with Luminex/xMAP technology)

as distributed in the network as possible

Stimulus   Perturbation

Readout   Perturb&Read

Differences between normal and transformed hepatocytes: targets for therapies?
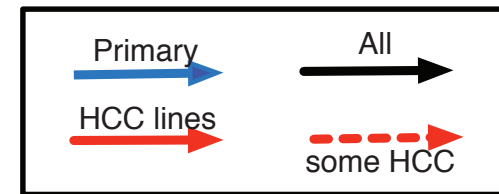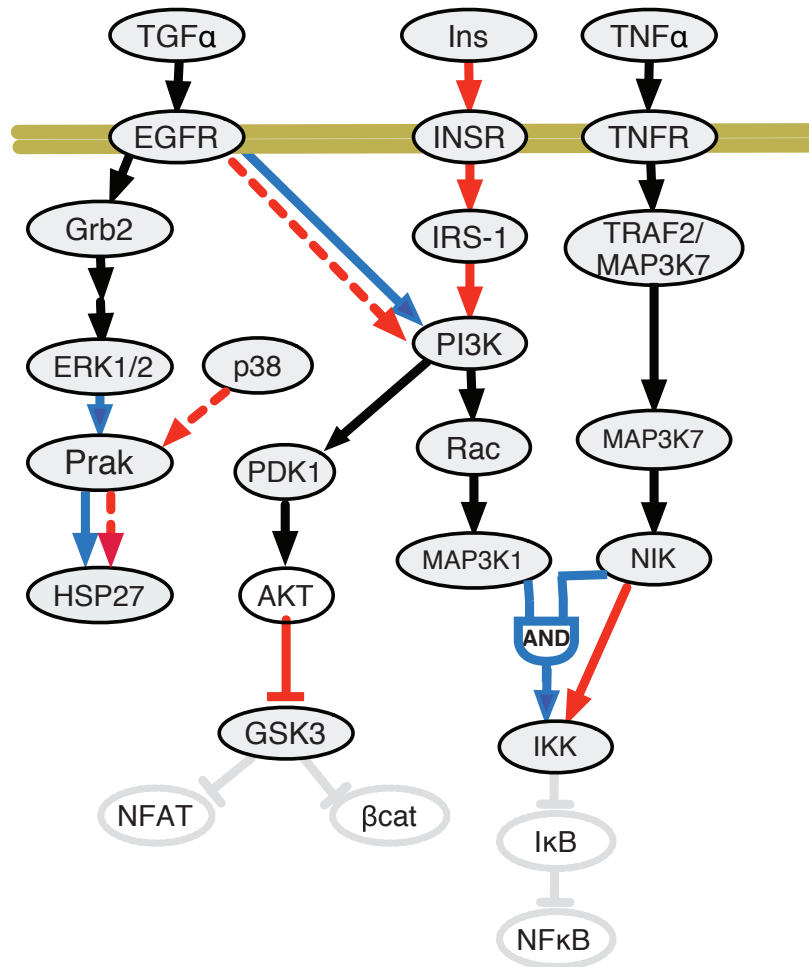
- Only active in HCC cell lines:
  Insulin→.. →AKT→GSK3

- HSP27 phosphorylation:
  ERK mediated in primary

- Difference in NFkB activation:
  TNF dependent only in HCC
  TNF+TGFα in primary
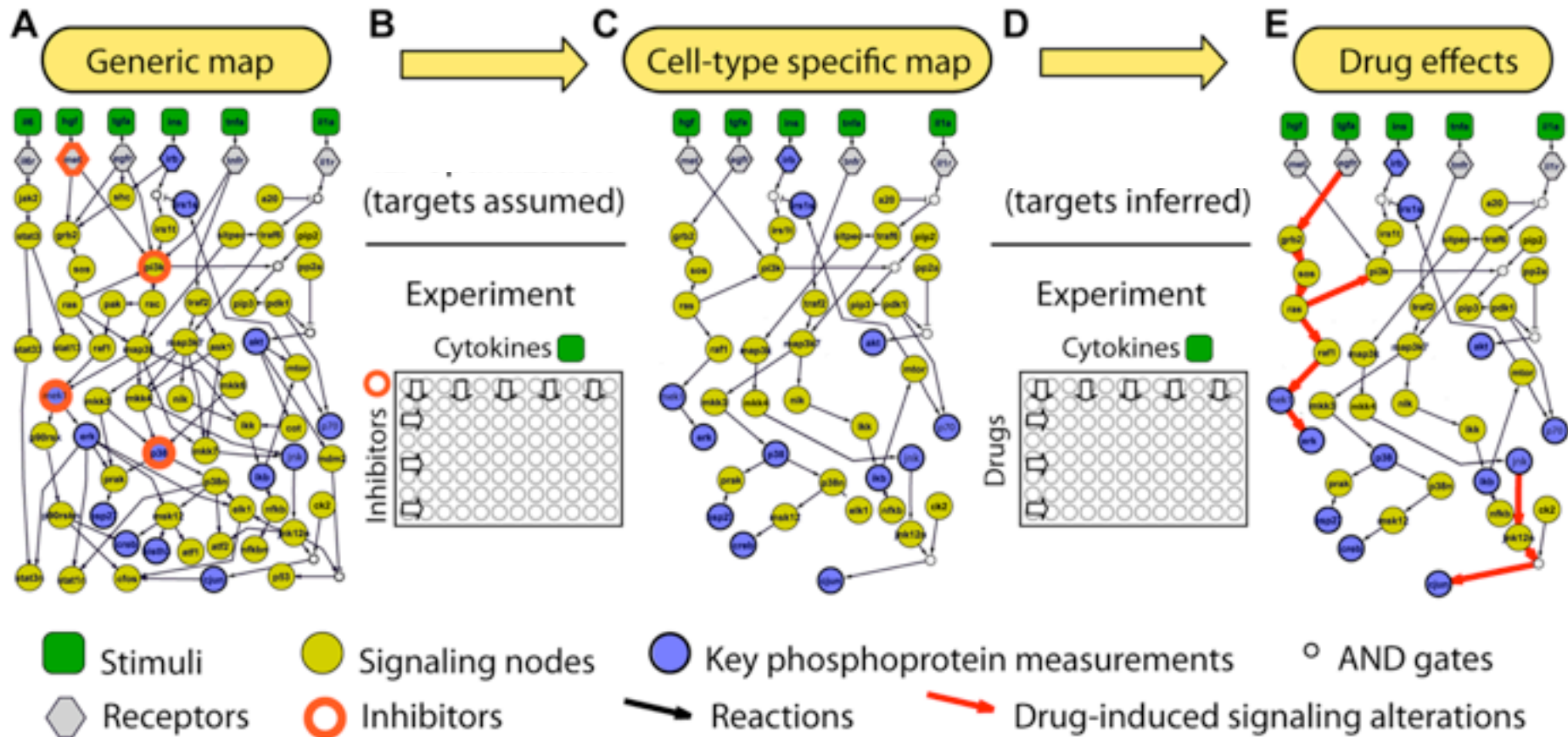
Saez-Rodriguez J, Alexopoulos LG, Zheng M, Lauffenburger DA, Sorger PK, *Cancer Research* 71(16) 1-12, 2011

Identification of off-target effect of Gefitinib (EGFR inhibitor) on IL1-alpha pathway (cJun activation)

Mitsos et al PLoS Comp Bio 2009

+ Precise characterization at biochemical level
- Limited scope (measurement limitations)
- No direct connection to phenotype
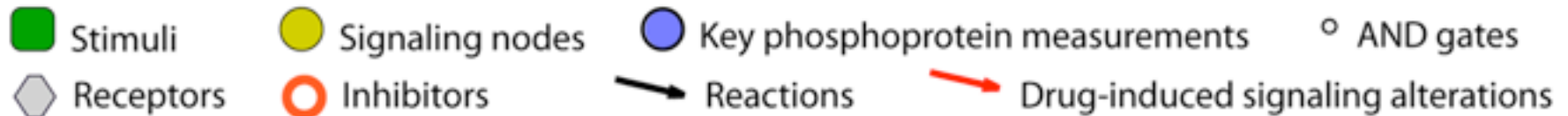
■ Stimuli	● Signaling nodes	● Key phosphoprotein measurements	° AND gates
⬡ Receptors	○ Inhibitors	→ Reactions	→ Drug-induced signaling alterations

Identification of off-target effect of Gefitinib (EGFR inhibitor) on IL1-alpha pathway (cJun activation)

Mitsos et al PLoS Comp Bio 2009

DNA

mRNA

Proteins

Network

Phenotype

Perturb with drugs

Measure changes in gene expression
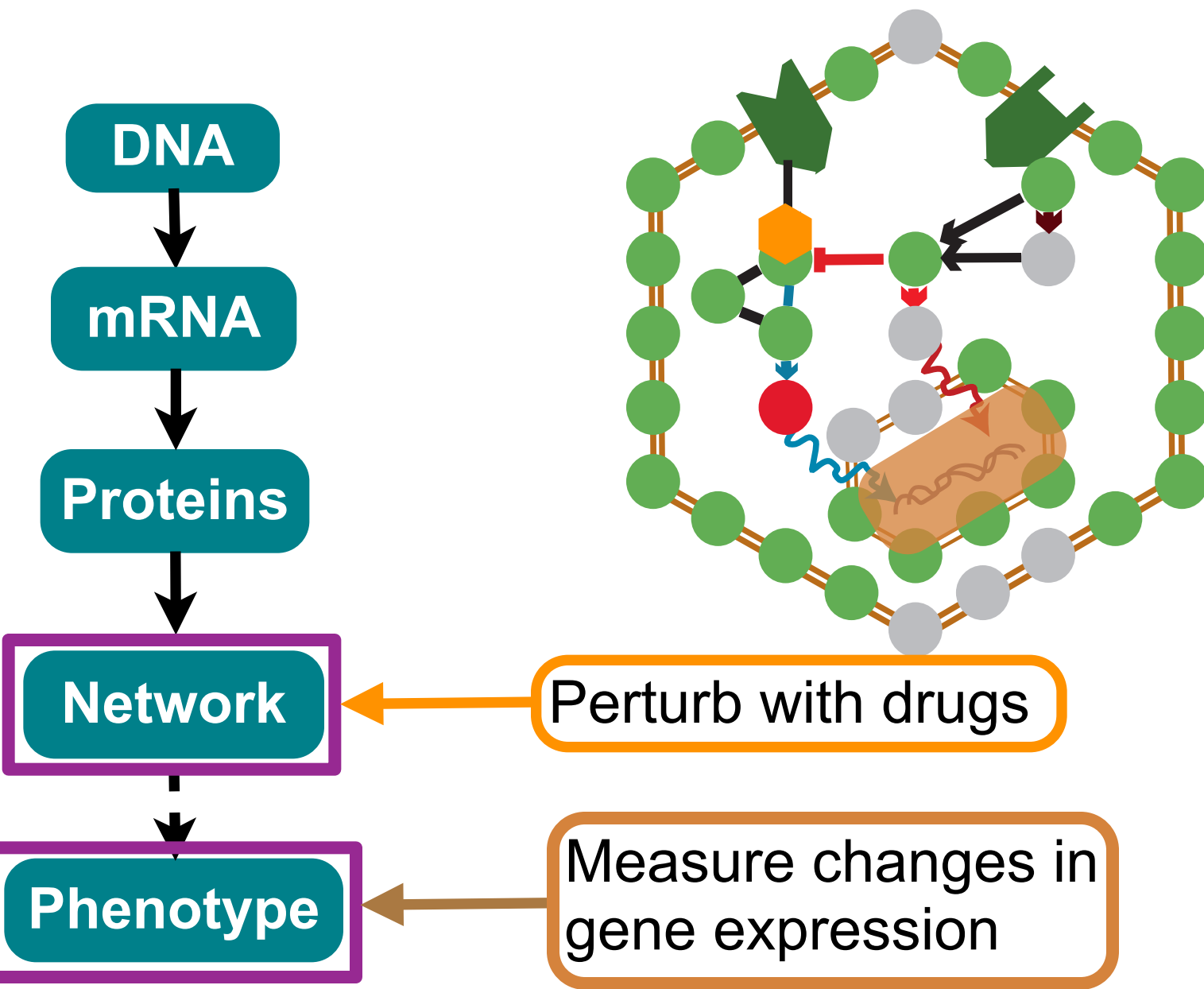
Use drug- & disease-induced transcriptional changes for drug discovery & repurposing
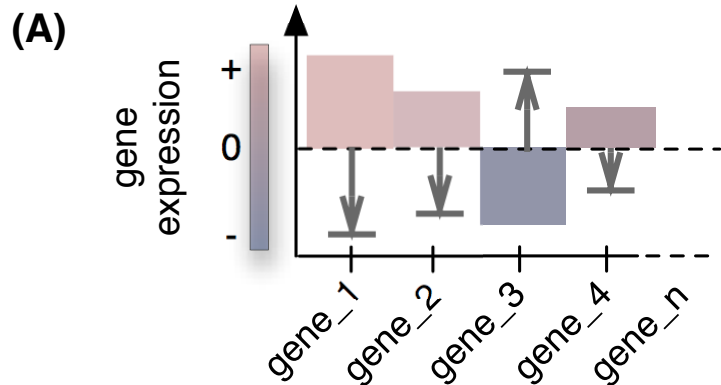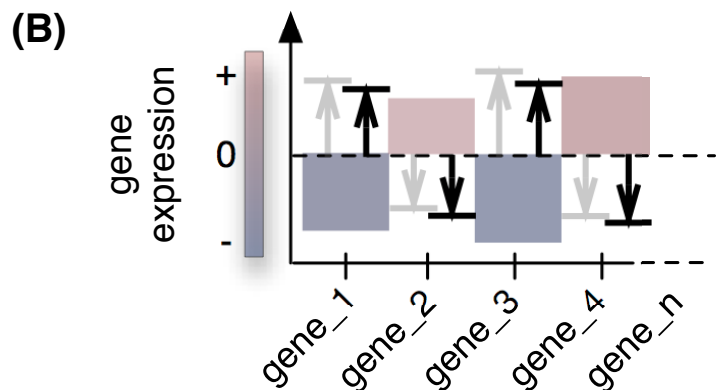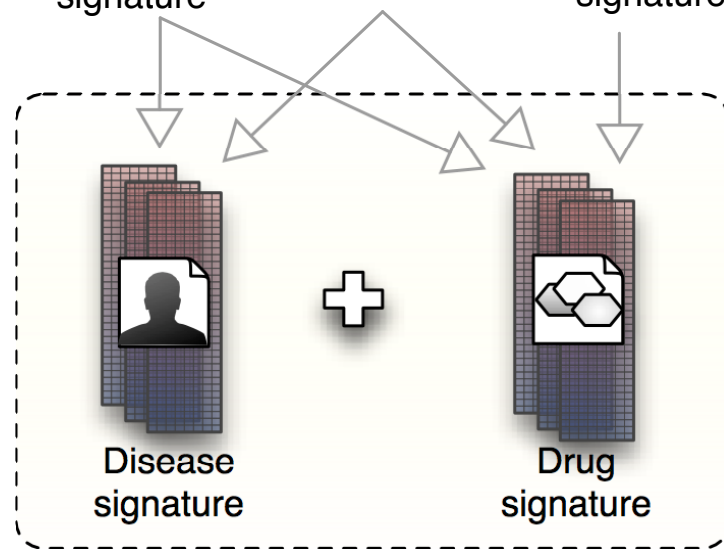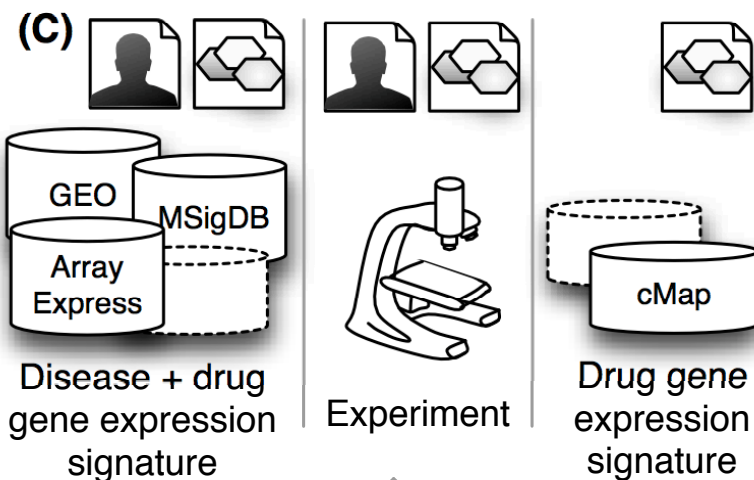
F Iorio
T Rittman H Ge
M Menden
J Saez-Rodriguez
Drug Discovery Today, in press
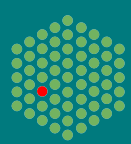
DvD: An R/Cytoscape pipeline for drug repurposing using public repositories of gene expression data

- Compare drug & disease signatures with dynamic access to databases (Array Express, GEO), and Connectivity Map
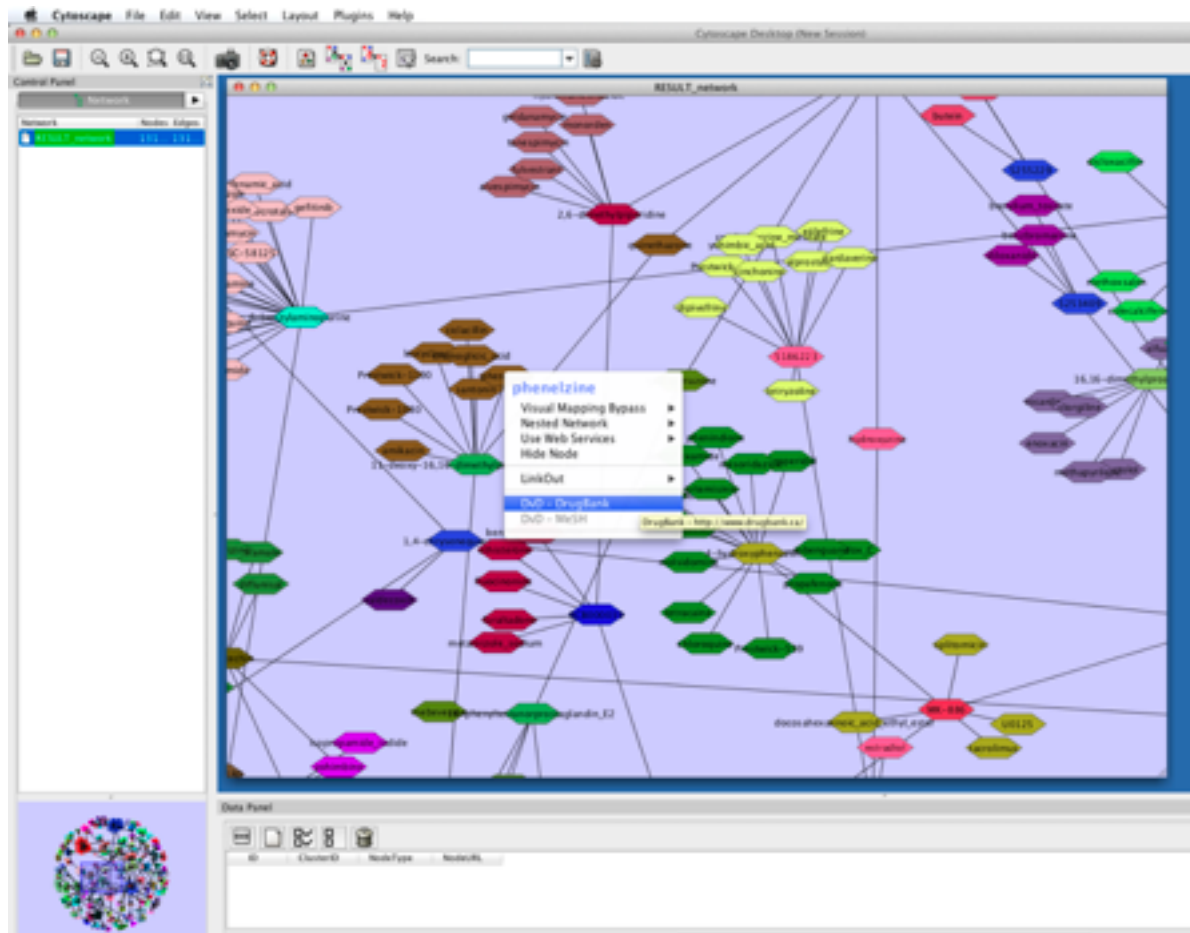
Pacini C Iorio F
Gonçalves E Iskar M
Klabunde T Bork P
Saez-Rodriguez J,
Bioinformatics,
2013

* Prednisolone = established compound for Crohn's disease
** Trinitobenzene Sulfonic Acid (TNBS)

Drug-disease score

-0.222   0   0.284

- 5186324
- ► **Topiramate**
- **Prednisolone**
- 12,13-EODE
- Tolbutamide
- Yohimbine
- 5213008
- Tomelukast
- Phenanthridinone
- 5162773
- 5151277
- Clotrimazole
- 5140203
- Genistein
- Fasudil
- 5230742
- 5182598

A

Vehicle only | TNBS + vehicle

TNBS + topiramate | TNBS + prednisolone

B

Gross pathology score

TNBS + veh | TNBS + pred | TNBS + top **** | Vehicle
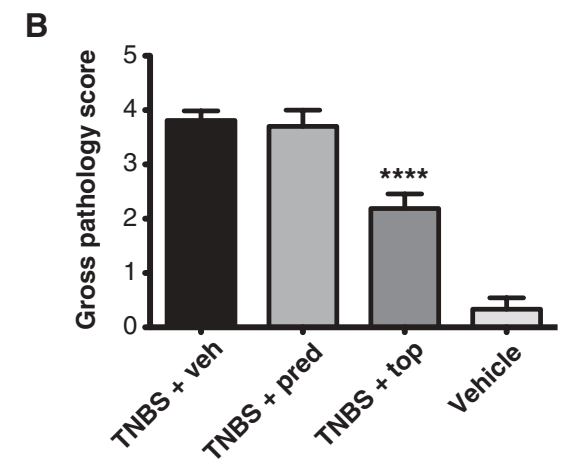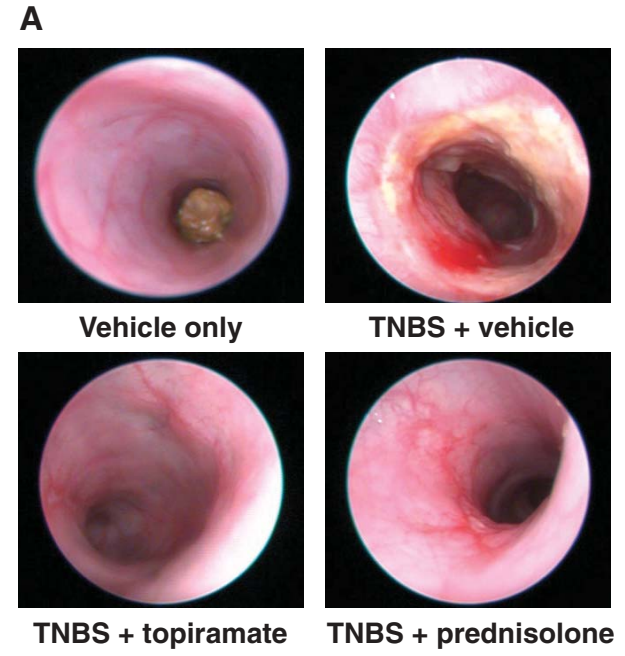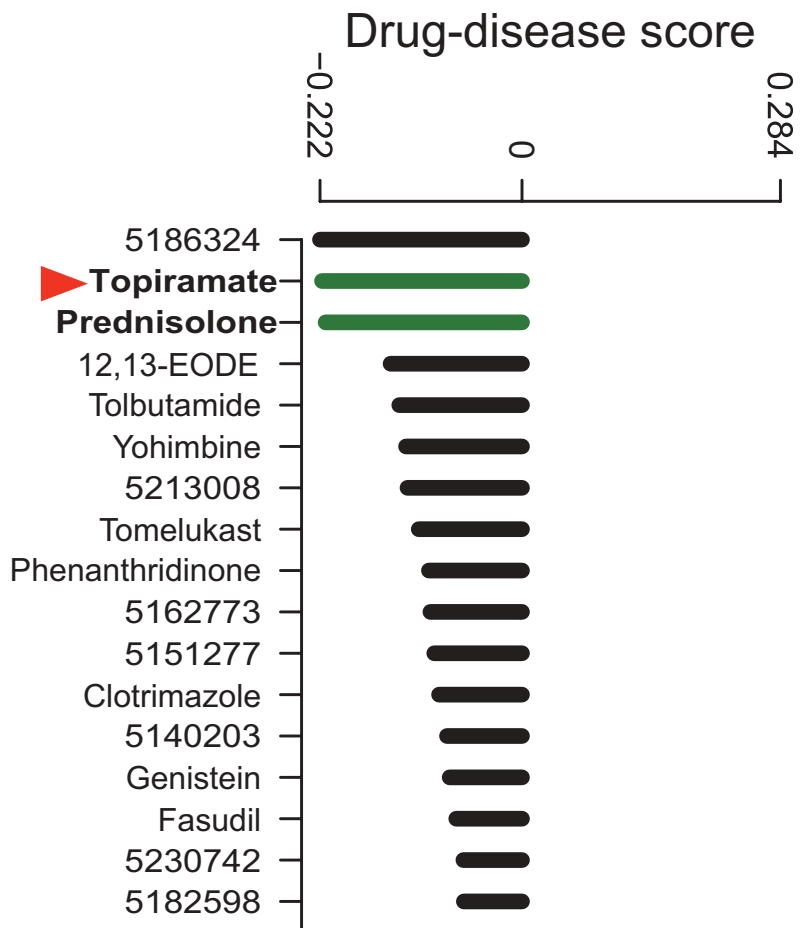
Computational Repositioning of the Anticonvulsant Topiramate for Inflammatory Bowel Disease
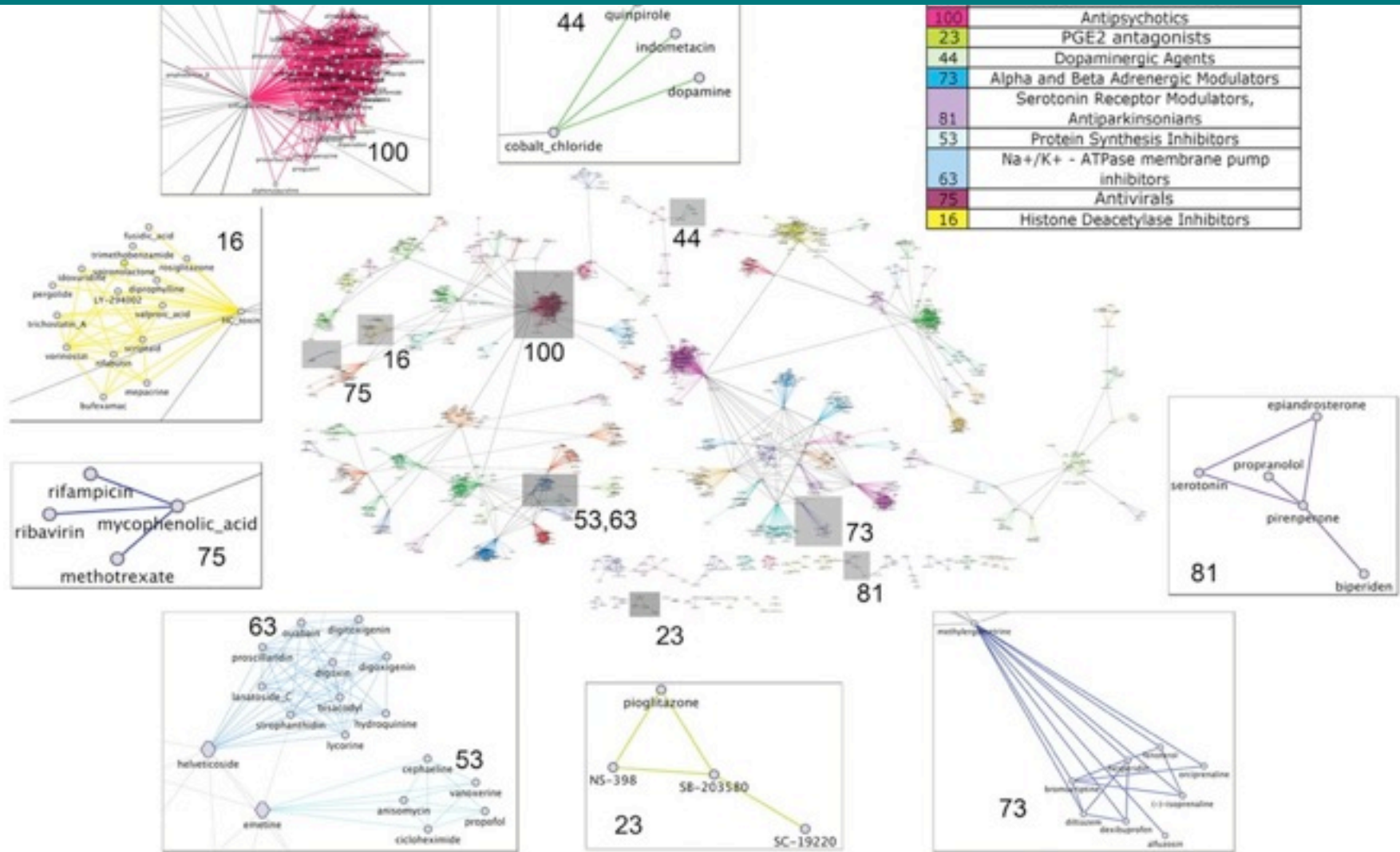
Discovery of drug mode of action and drug repositioning from transcriptional responses Iorio et al, PNAS 2010
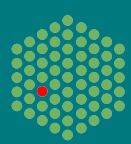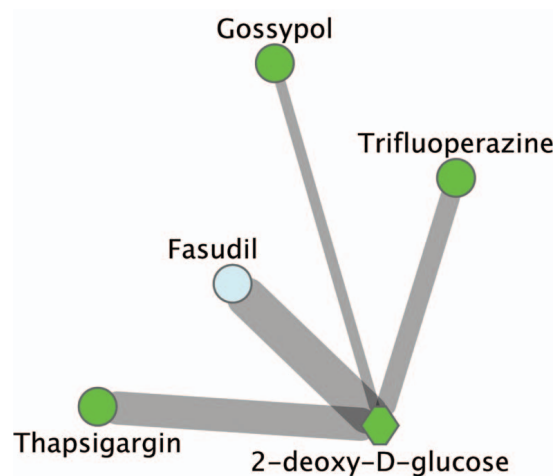
2DOG neighborhood

1	Fasudil	0.5162
2	Thapsigargi	0.5644
3	Trifluoperaz	0.577
4	Gossypol	0.633
5	Niclosamid	0.6539
...	...	...
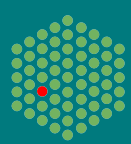
Identification of small molecules enhancing autophagic function from drug network analysis

Iorio et al, Autophagy 2010
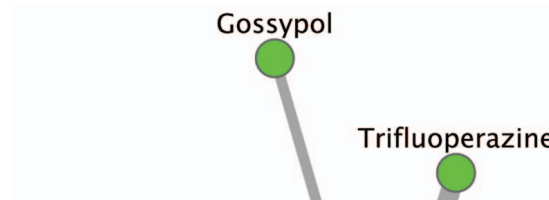
+ Genome-wide characterization
+ Based on 'phenotype'
- None or limited mechanistic understanding
- No direct connection to phenotype

Identification of small molecules enhancing autophagic function from drug network analysis

Iorio et al, Autophagy 2010

From cancer drug-responses to signaling pathways

DNA → mRNA → Proteins → Network → Phenotype
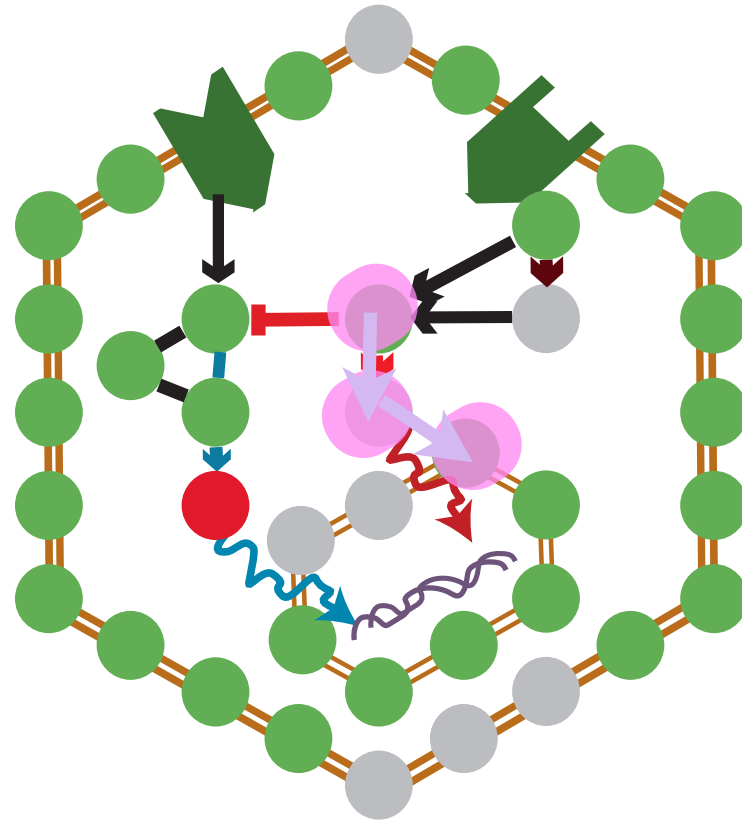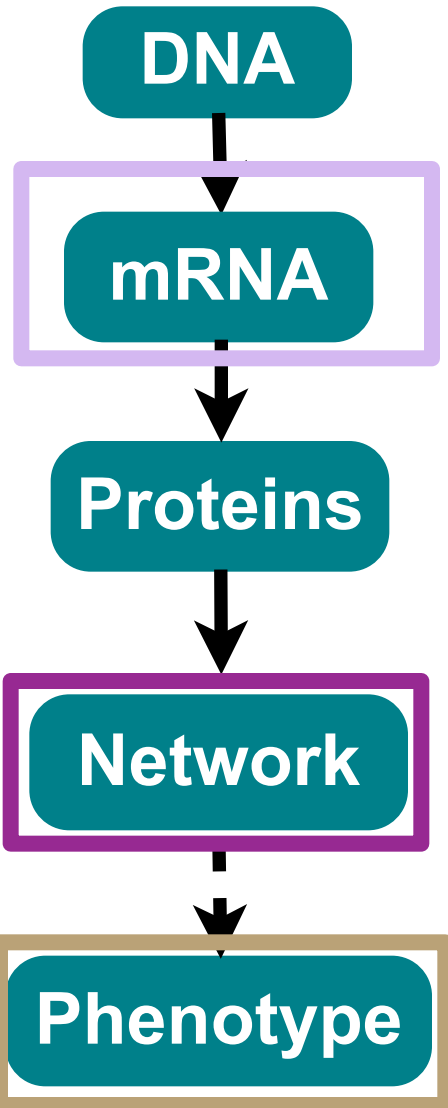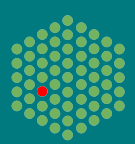
- Map expression on pathways?

DNA

mRNA

Proteins

Network

Phenotype
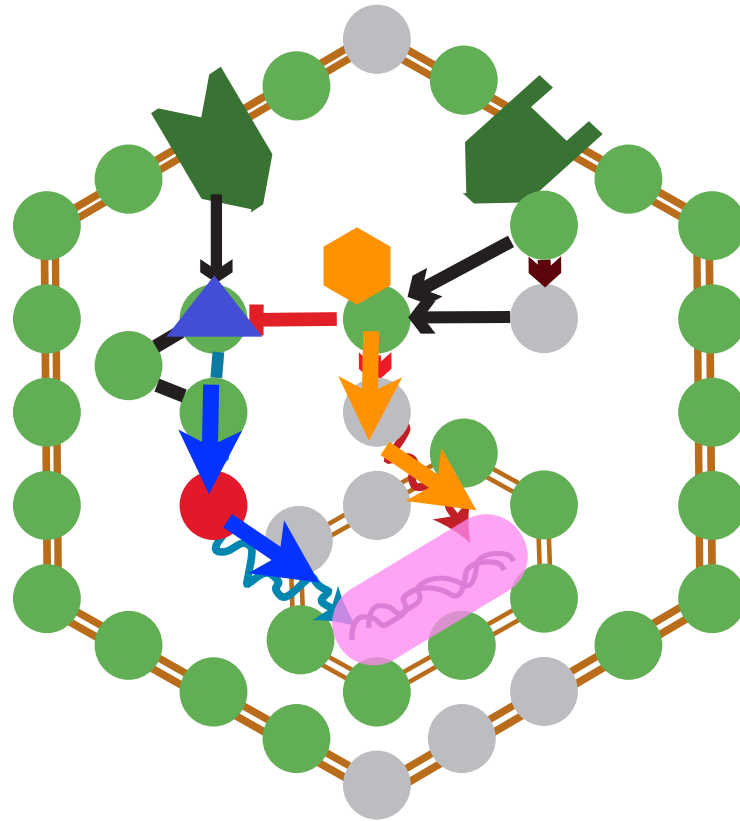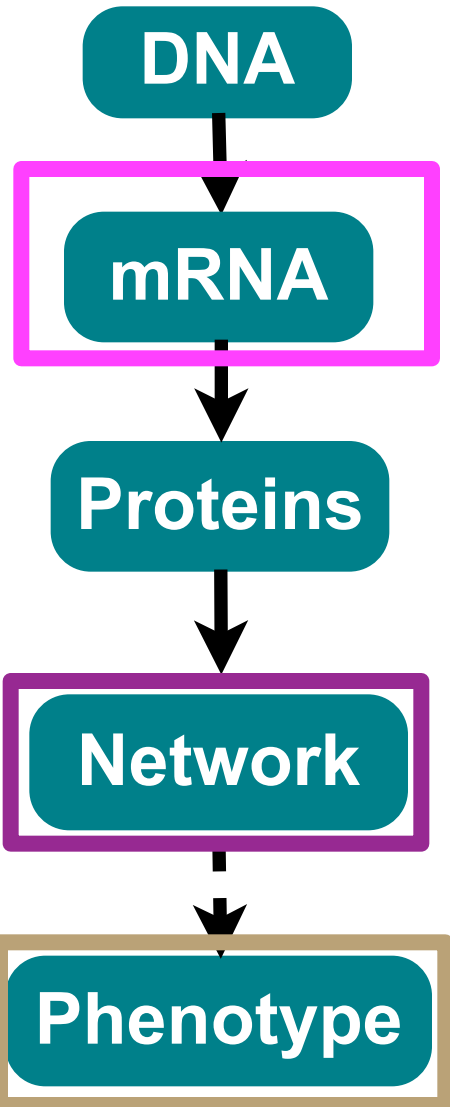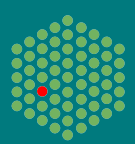
1-Identify transcription factors involved in drug's mode of action

2- Find pathways linking the transcription factors to the drug targets

An integer linear programming approach for finding deregulated subgraphs in regulatory networks

Christina Backes[1,*], Alexander Rurainski[2,*], Gunnar W. Klau[3], Oliver Müller[4], Daniel Stöckel[4], Andreas Gerasch[5], Jan Küntzer[6], Daniela Maisel[6], Nicole Ludwig[1], Matthias Hein[7], Andreas Keller[1,8], Helmut Burtscher[9], Michael Kaufmann[5], Eckart Meese[1] and Hans-Peter Lenhof[4]
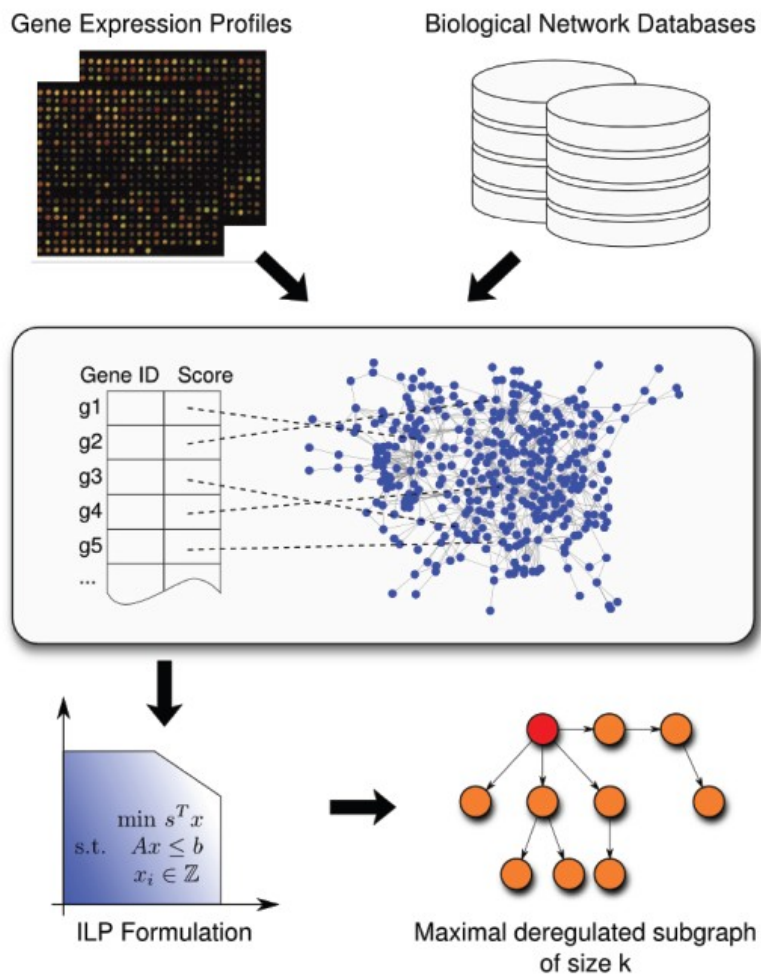
BMC
Genomics
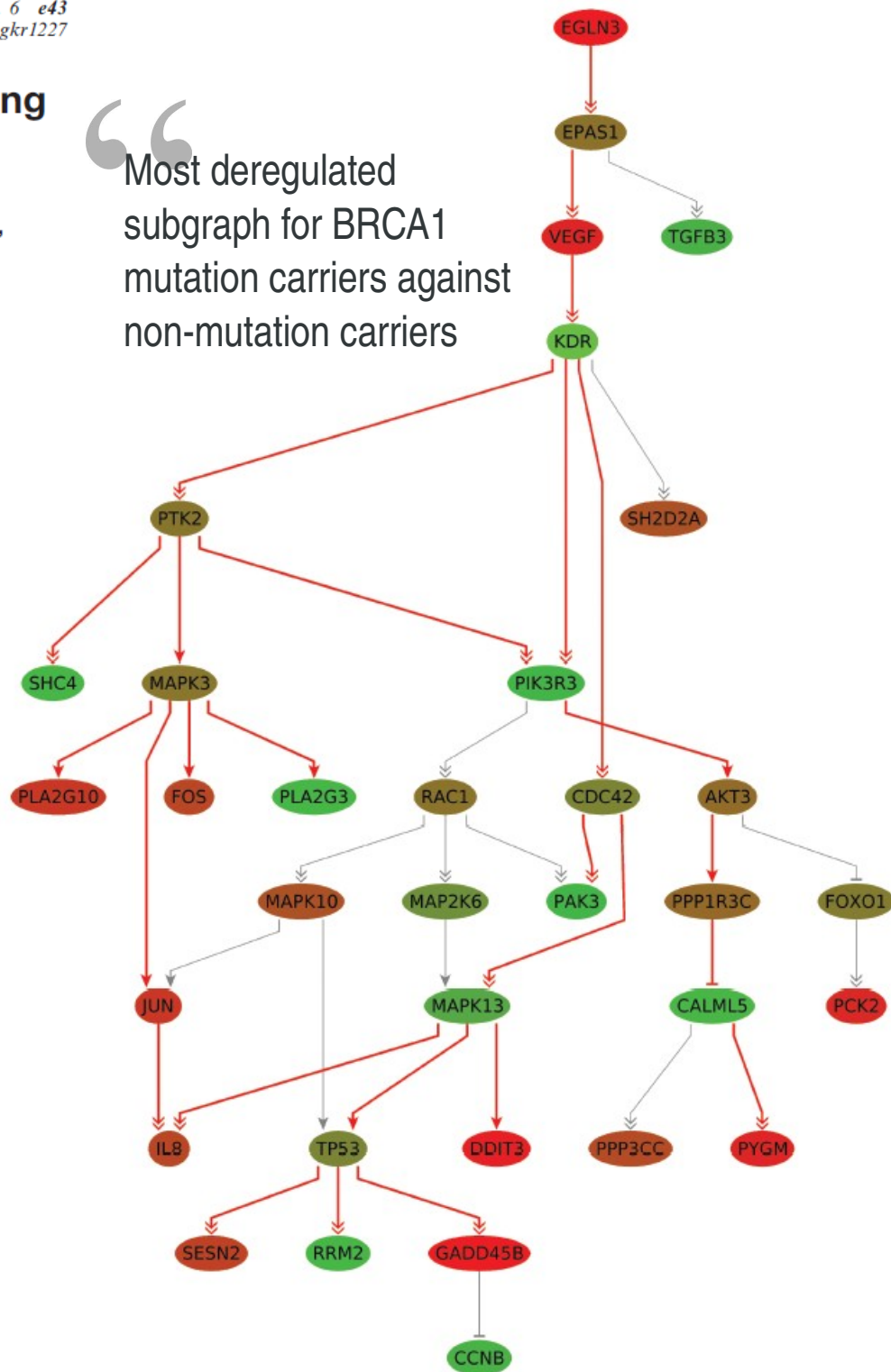
Beyond differential expression: the quest for causal mutations and effector molecules

Nicholas J Hudson[*], Brian P Dalrymple and Antonio Reverter
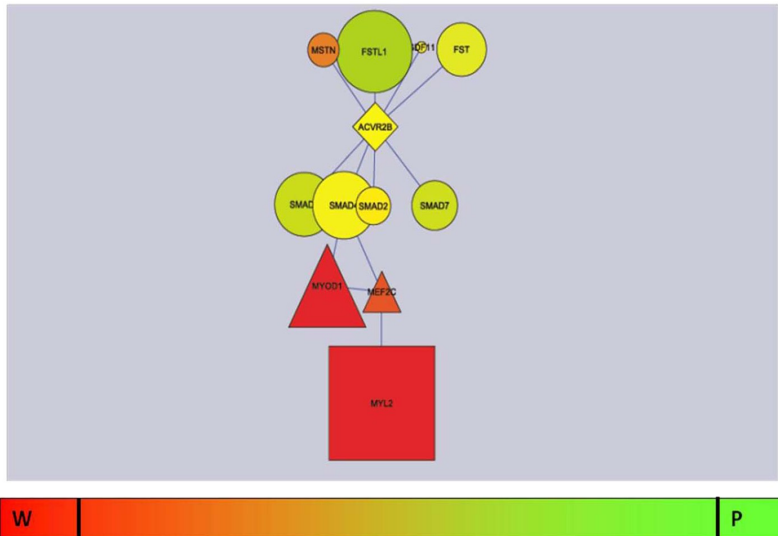
> " Furthermore, a very recent review [40] states that gene set enrichment analyses are "…commonly applied to identify enrichment of biological functional categories in sets of ranked differentially expressed genes from genome-wide mRNA expression data sets."
>
> Abundance, abundance, abundance! So, how does one get a measure of a molecule's behaviour?

→ Transcription Factor activity does not need to correlate with their abundance

(they just leave a "fingerprint" on the expression profile)

BMC Genomics

Beyond differential expression: the quest for causal mutations and effector molecules

Nicholas J Hudson*, Brian P Dalrymple and Antonio Reverter

Differential co-expression

MYL2: muscle structural protein
MSTN: negative regulator of muscle mass

Figure: mutant (left) vs. wild-type (right)

Drawback: -does not know if interaction direct or indirect
-need to know that MSTN is perturbed
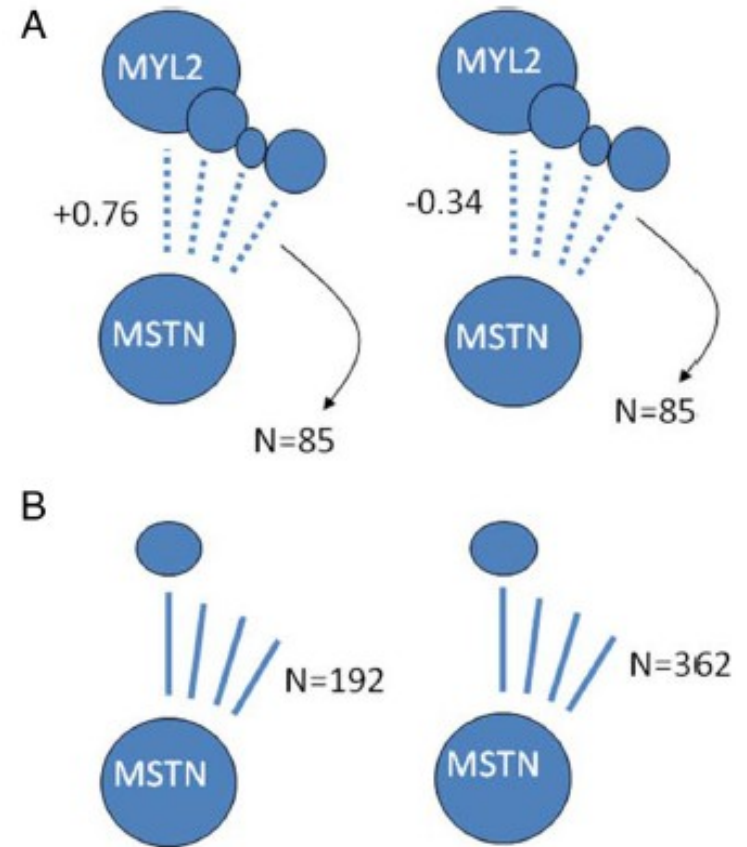
Figure 5 MSTN is highly differentially co-expressed with many of the abundant, highly differentially expressed genes - mutant breed on the left, wildtype breed on the right. For example, MSTN has a differential co-expression of 1.1 (+0.76 - - 0.34) with MYL2 (Panel **A**). RIF accumulates these differential co-expressions for all the DE genes (85 in this instance), weighted by their abundance. The size of the bubble representing the various DE genes corresponds to the combination of the extent of DE and average abundance. An alternative measure of differential connectivity is given in Panel **B**, where the number of significant co-expressions possessed by MSTN in the two breeds is contrasted. MSTN does not get prioritised by this alternative approach.

Reverse engineering of regulatory networks in human B cells

Katia Basso[1], Adam A Margolin[2], Gustavo Stolovitzky[3], Ulf Klein[1], Riccardo Dalla-Favera[1,4] & Andrea Califano[2]
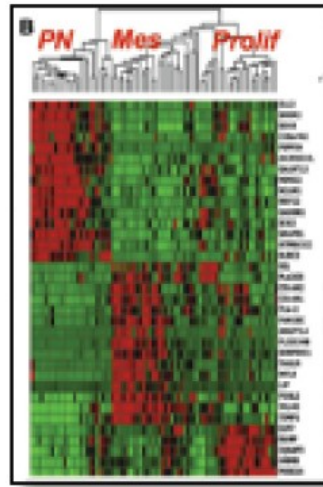
Mesenchymal Signature of High Grade Glioma

ARACNe MRA →
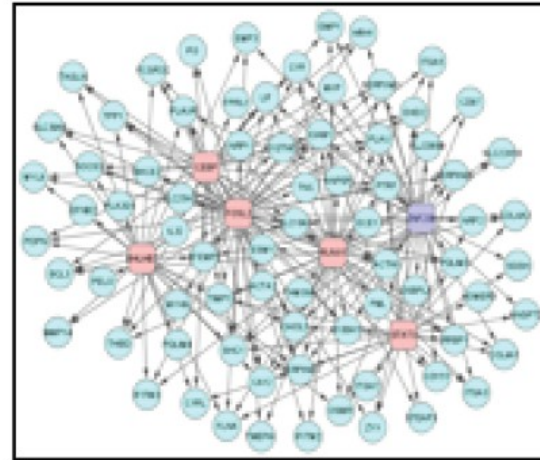
Regulatory Network

336 expression profiles representative of perturbations of B cell phenotypes

eliminates indirect interactions as opposed to co-expression methods

c Unconditional (ARACNe)
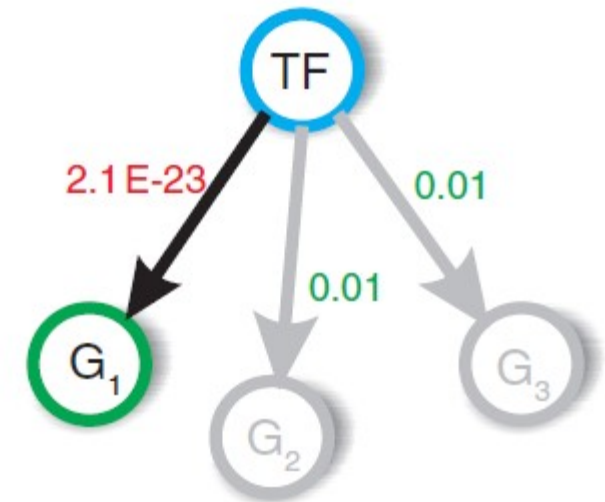
(images from Carro2009 and Wang2009)

Genome-wide identification of post-translational modulators of transcription factor activity in human B cells

Kai Wang[1,2,5,6], Masumichi Saito[3,5,6], Brygida C Bisikirska[2], Mariano J Alvarez[2], Wei Keat Lim[1,2,5], Presha Rajbhandari[2], Qiong Shen[3], Ilya Nemenman[2,5], Katia Basso[3], Adam A Margolin[1,2,5], Ulf Klein[3], Riccardo Dalla-Favera[3,4] & Andrea Califano[1-3]
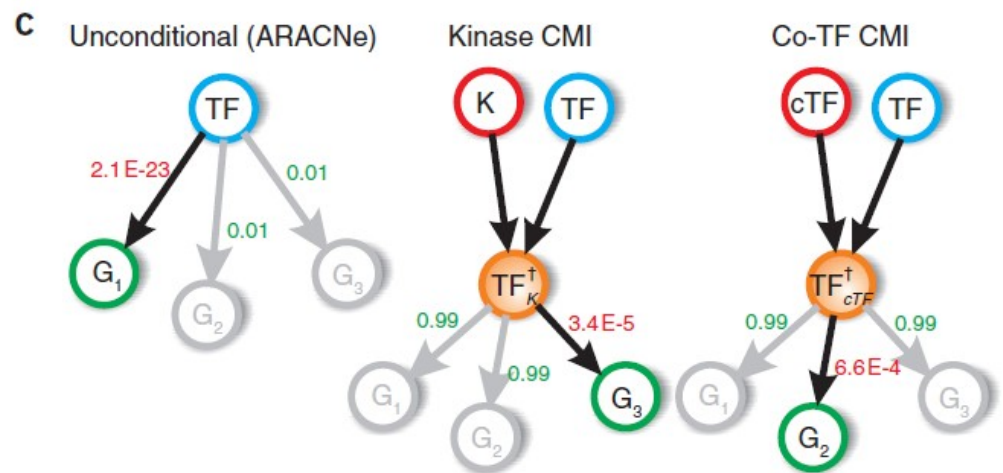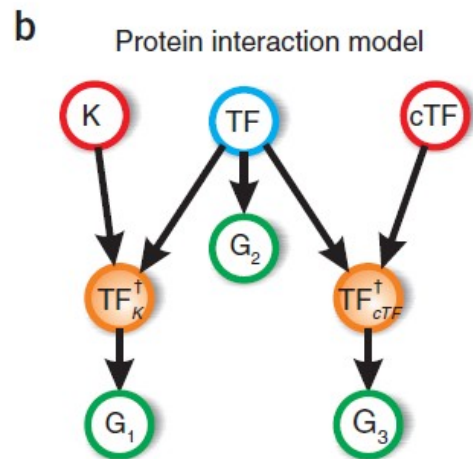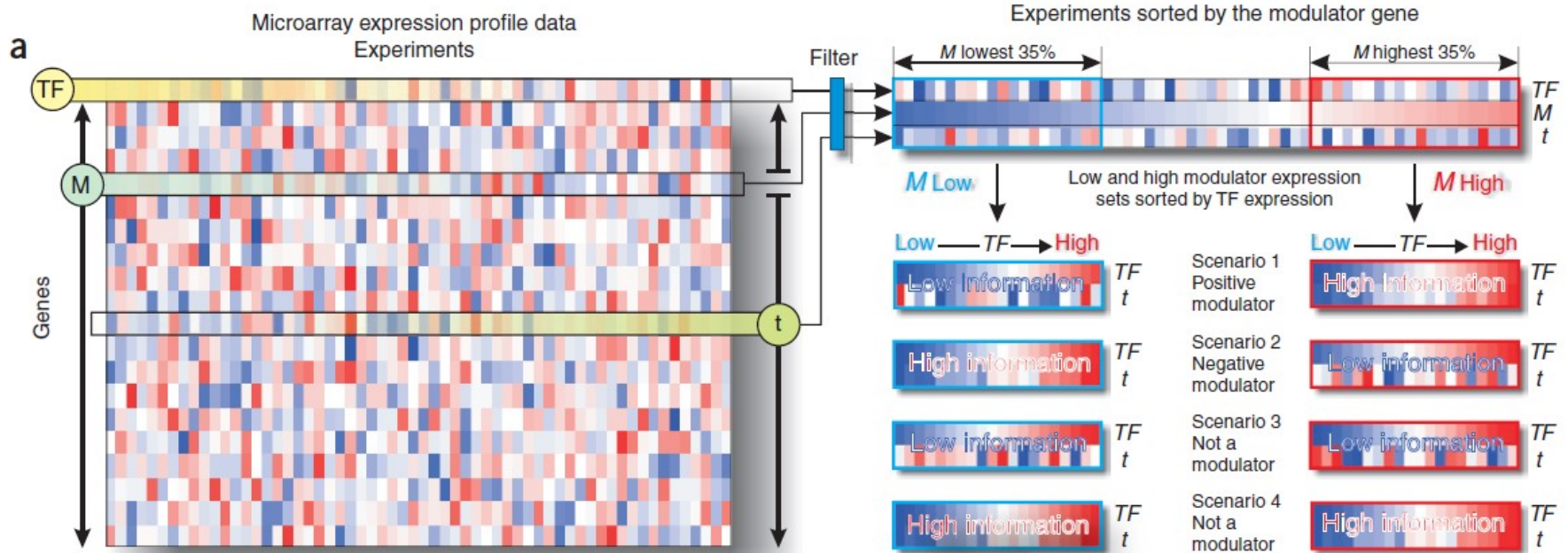
Inferring TF activity from expression

is like inferring the stones
from the ripples in a pond

molecular
systems
biology

A human B-cell interactome identifies MYB and FOXM1 as master regulators of proliferation in germinal centers
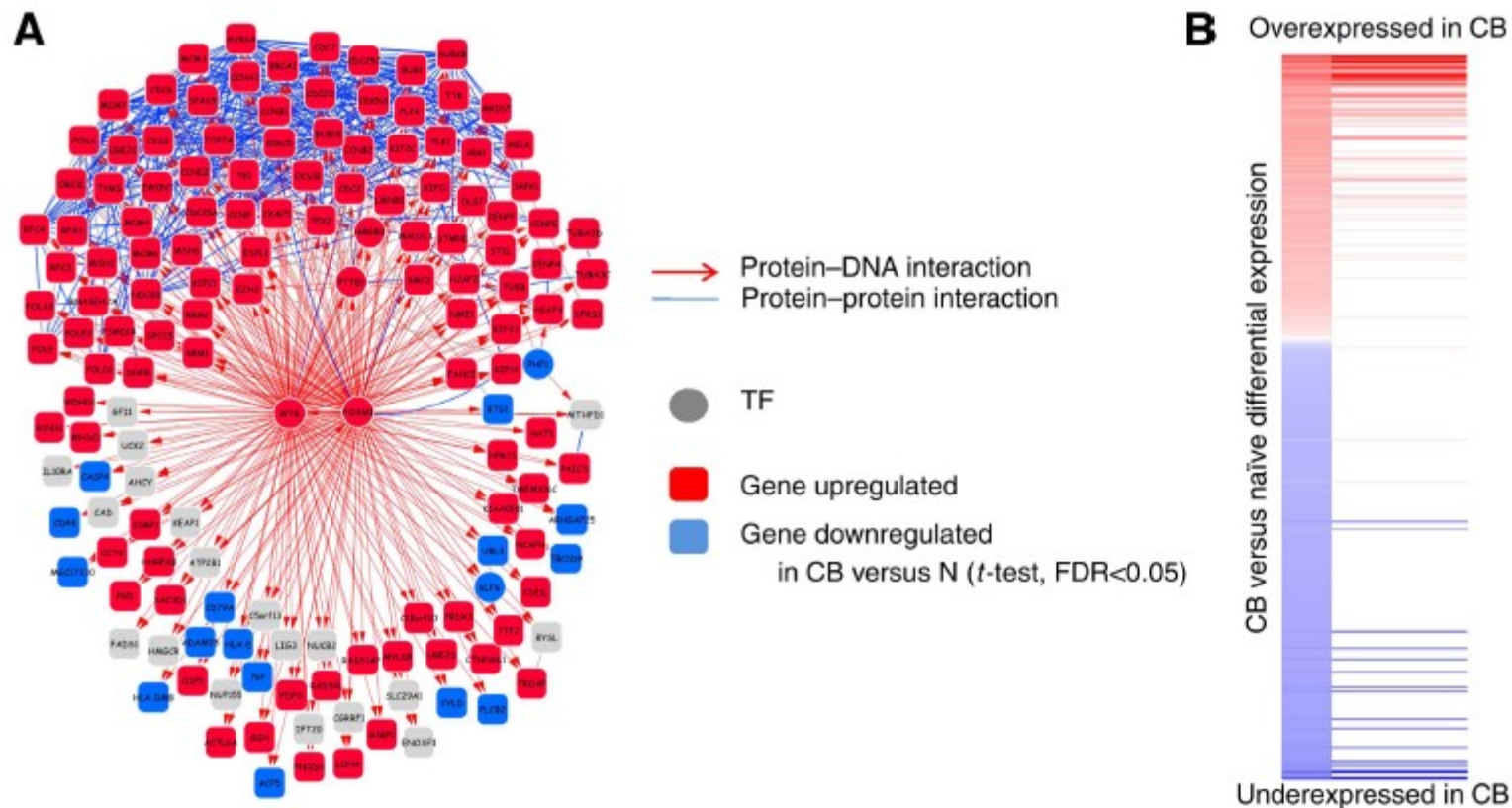
Identify TF activity by GSEA of its regulon (GSEA intro: http://goo.gl/zOmtJ)

Causal reasoning on biological networks: interpreting transcriptional changes

Leonid Chindelevitch[1,†], Daniel Ziemek[1,*,†], Ahmed Enayetallah[2], Ranjit Randhawa[1],
Ben Sidders[3], Christoph Brockel[4] and Enoch S. Huang[1]
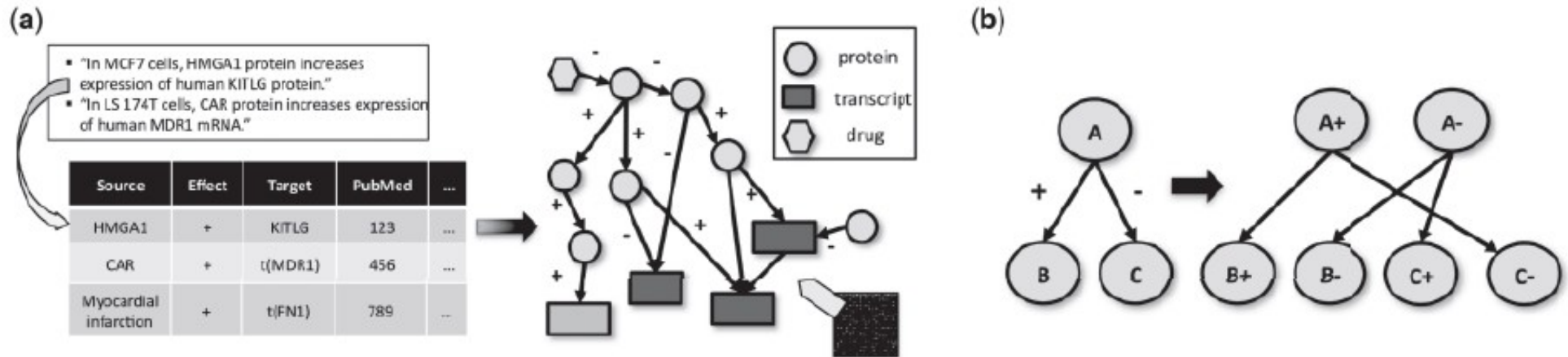
Example of using qualitative statements for reasoning on a graph

Biological follow-up study:

OPEN ACCESS Freely available online

PLoS one

Modeling the Mechanism of Action of a DGAT1 Inhibitor Using a Causal Reasoning Platform

Ahmed E. Enayetallah[1*], Daniel Ziemek[2], Michael T. Leininger[3], Ranjit Randhawa[2], Jianxin Yang[3], Tara B. Manion[3], Dawn E. Mather[3], William J. Zavadoski[3], Max Kuhn[3], Judith L. Treadway[3], Shelly Ann G. des Etages[3], E. Michael Gibbs[3], Nigel Greene[1], Claire M. Steppan[3]

Method

Revealing signaling pathway deregulation by using gene expression signatures and regulatory motif analysis

Yingchun Liu[*] and Markus Ringnér[†]

Addresses: *Computational Biology and Biological Physics, Department of Theoretical Physics, Lund University, SE-221 85, Sweden. †Division of Oncology, Department of Clinical Sciences, Lund University, SE-221 85, Sweden.

Correspondence: Markus Ringnér. Email: markus.ringner@med.lu.se

(a) Information retrieval
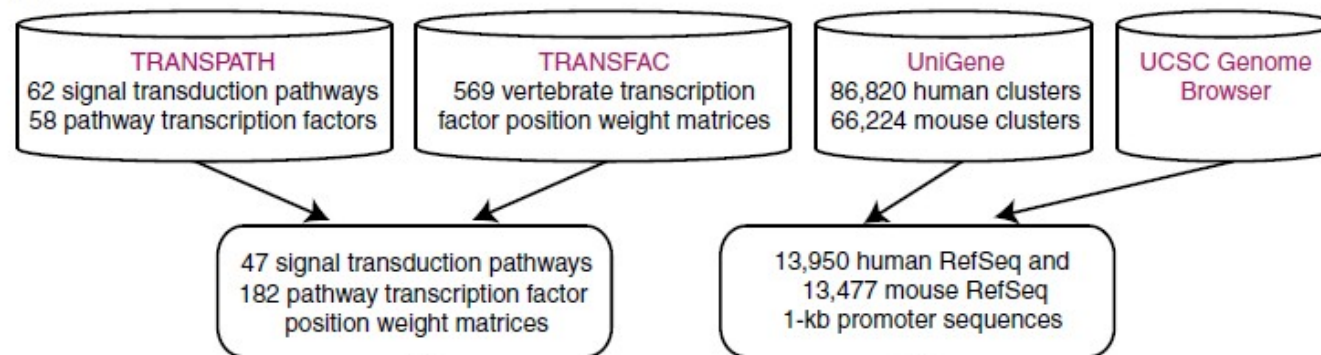
(b) Pathway deregulation analysis procedure
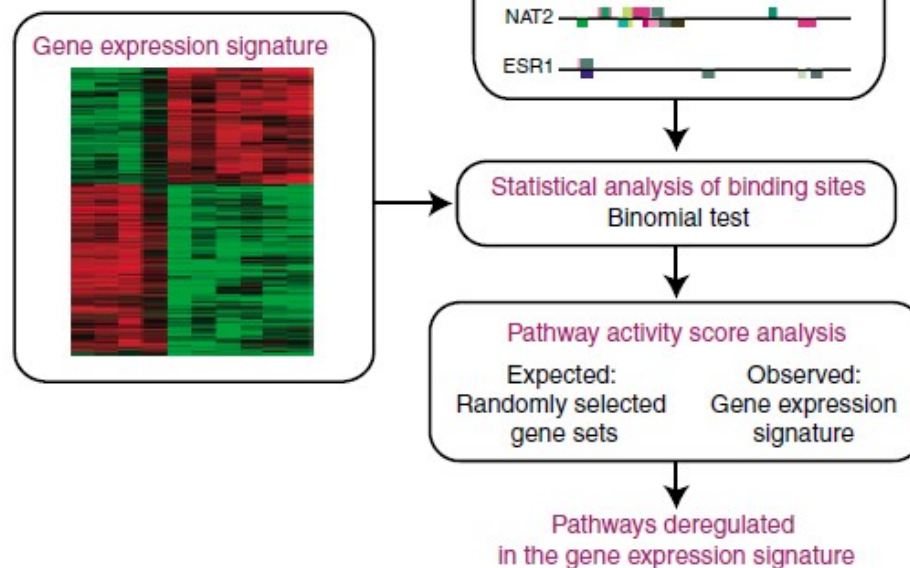
An alternative: Nested Effects Models
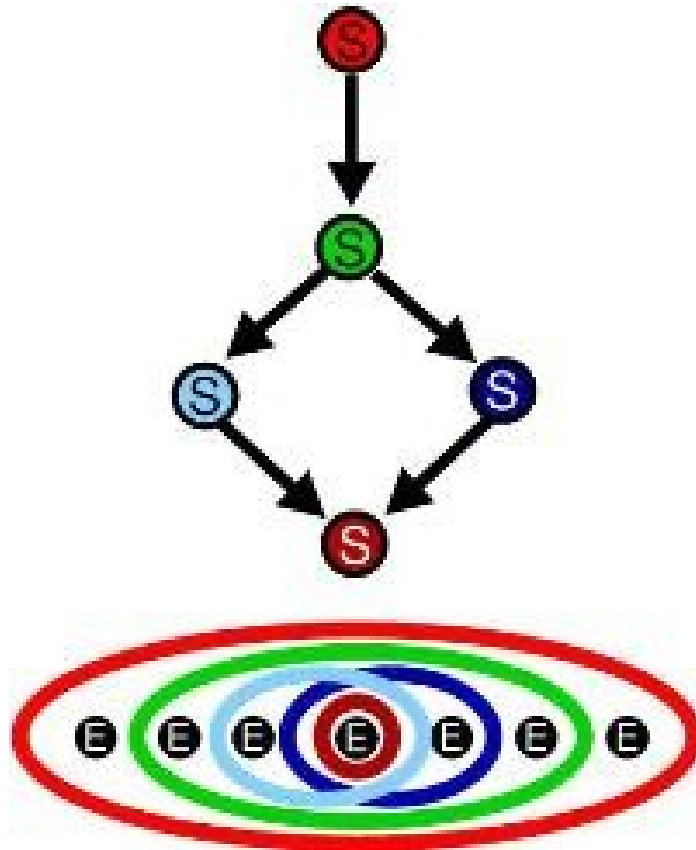
RNAi or drug perturbations

Reasoning on transcriptional data

- * Hudson, N.J., Dalrymple, B.P. & Reverter, A., 2012. Beyond differential expression: the quest for causal mutations and effector molecules. BMC Genomics, 13(1), p.356. (review)

- *** Chindelevitch, L. et al., 2012. Causal reasoning on biological networks: interpreting transcriptional changes. Bioinformatics, 28(8), pp.1114–1121.

- * Gosline, S.J.C. et al., 2012. SAMNet: a network-based approach to integrate multi-dimensional high throughput datasets. Integrative Biology.

- ** Silberberg, Y. et al., 2012. Large-Scale Elucidation of Drug Response Pathways in Humans. Journal of Computational Biology, 19(2), pp.163–174.

- *** Lefebvre, C. et al., 2010. A human B-cell interactome identifies MYB and FOXM1 as master regulators of proliferation in germinal centers. Molecular Systems Biology, 6.

- * Pe'er, D. & Hacohen, N., 2011. Principles and Strategies for Developing Network Models in Cancer. Cell, 144(6), pp.864–873. (review; strategy 3 and 6 are most interesting)

- ** Enayetallah, A.E. et al., 2011. Modeling the Mechanism of Action of a DGAT1 Inhibitor Using a Causal Reasoning Platform. PLoS ONE, 6(11), p.e27009.

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