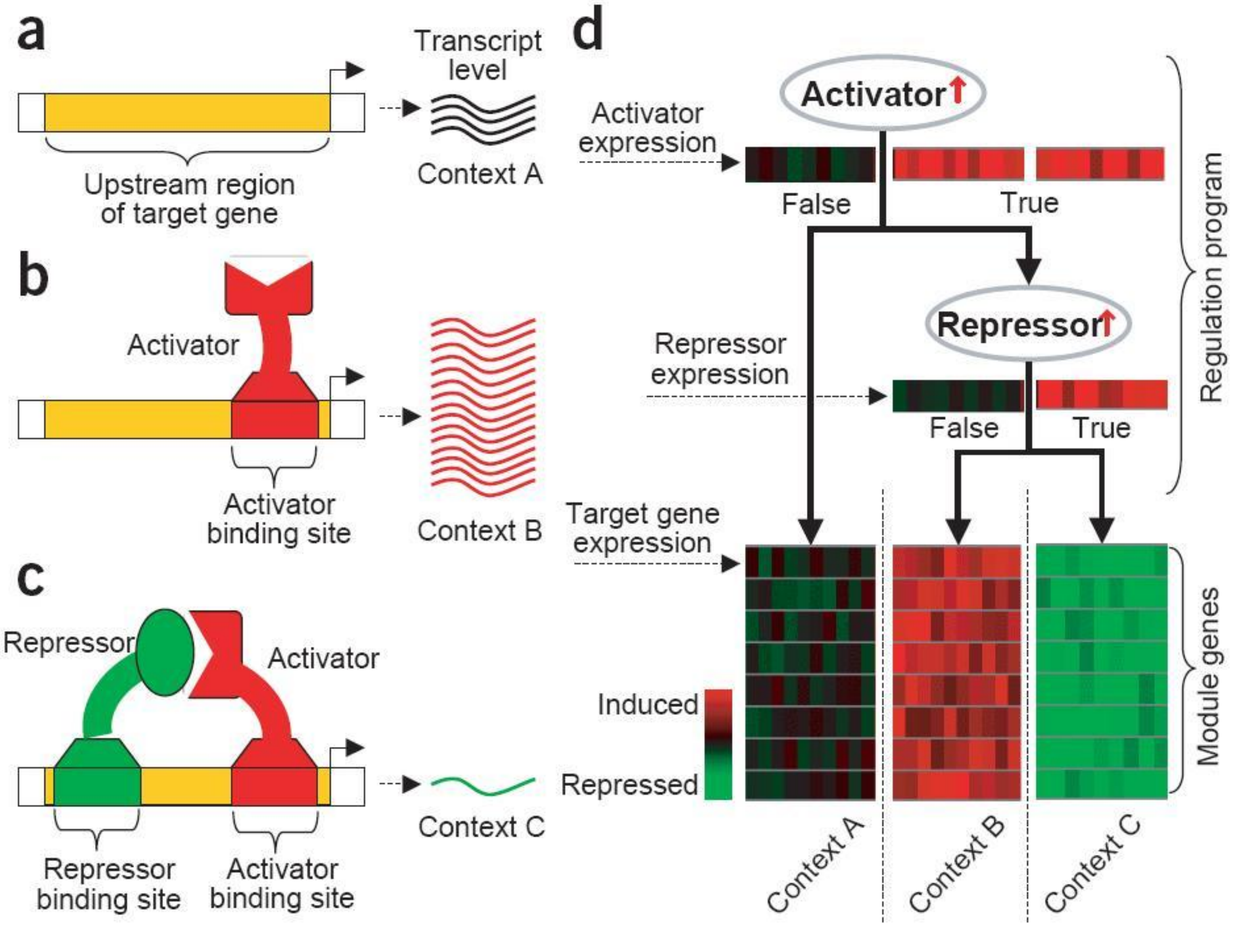
Inferring regulatory, signaling & metabolic networks from data

Devika Subramanian
Comp 470

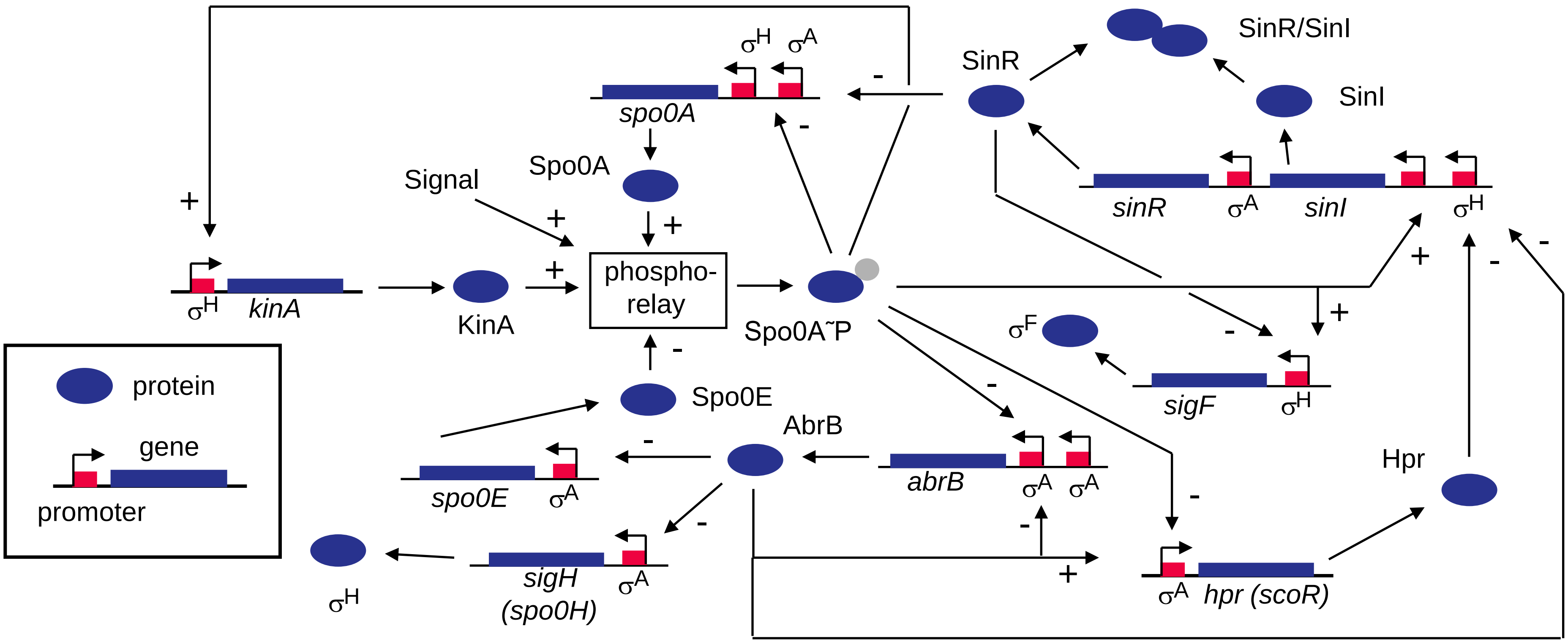
Networks

- Regulatory network: network of control decisions used to turn genes on/off.
- Signaling network: interactions among genes, gene products and small molecules that activate cellular processes.
- Metabolic network: network of proteins that synthesize and breakdown cellular molecules.

Regulators


a
Transcript level
Context A
Upstream region of target gene
b
Activator
Activator binding site
Context B
c
Repressor
Activator
Repressor binding site
Activator binding site
Context C
d
Activator
Activator expression
False
True
Repressor
Repressor expression
False
True
Regulation program
Target gene expression
Induced
Repressed
Module genes
Context A
Context B
Context C

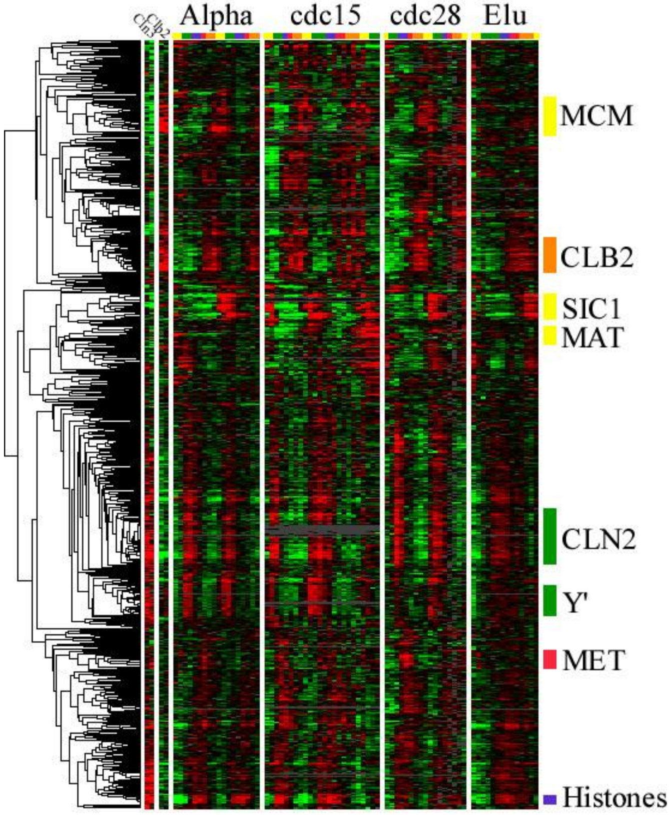
Genetic regulatory network of *B. subtilis*



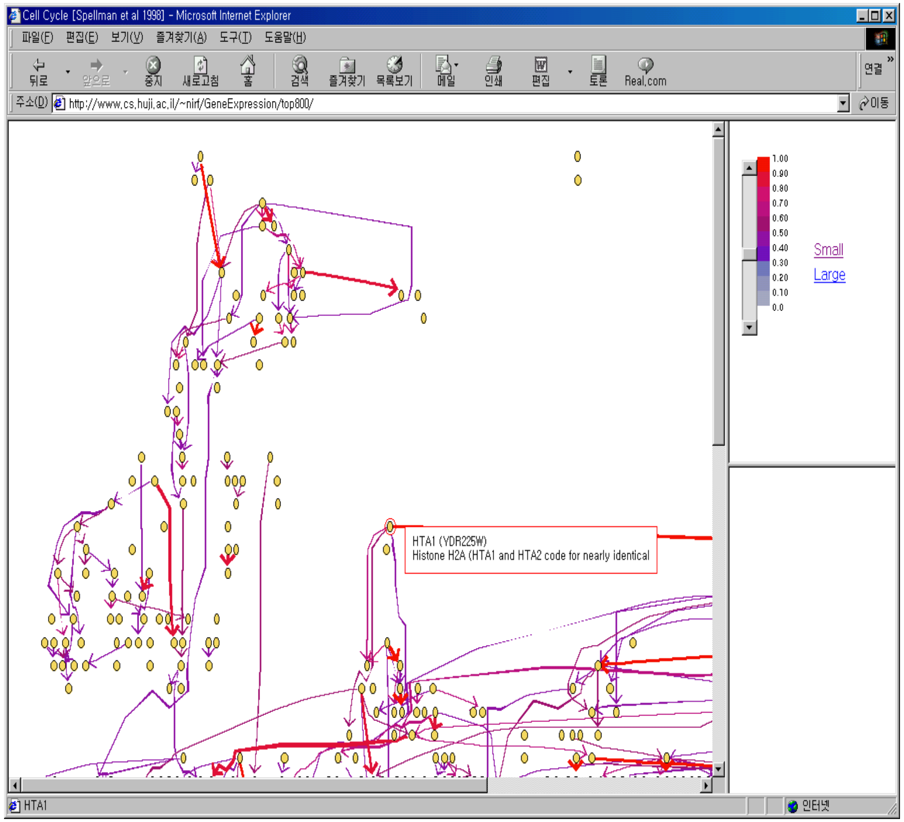
Genetic regulatory network controlling the initiation of sporulation.

(c) Devika Subramanian, 2009

From expression data to gene regulatory networks



Microarray data

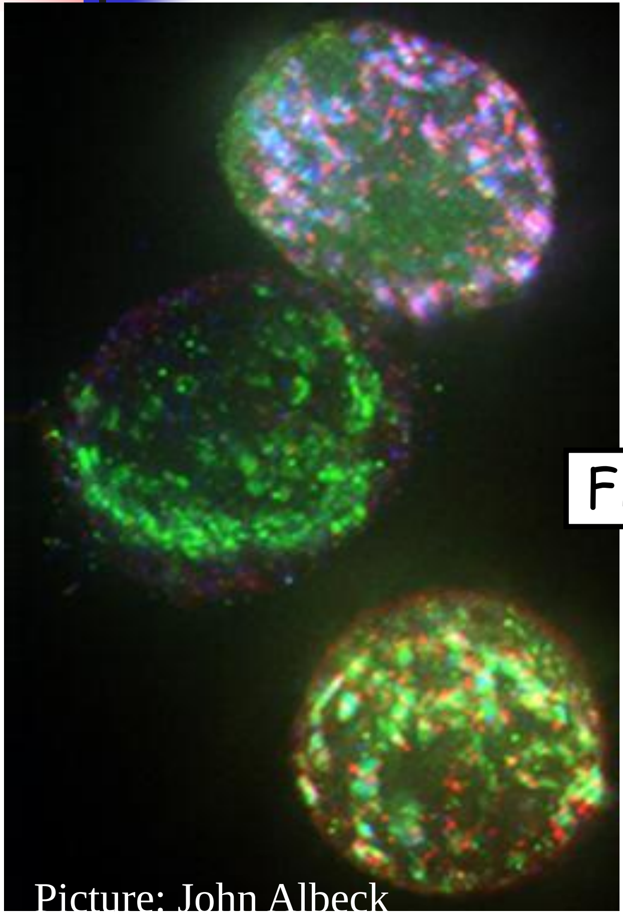

Cell Cycle [Spellman et al 1998] - Microsoft Internet Explorer

http://www.cs.huji.ac.il/~nirf/GeneExpression/top800/

HTA1 (YDR225W)
Histone H2A (HTA1 and HTA2 code for nearly identical

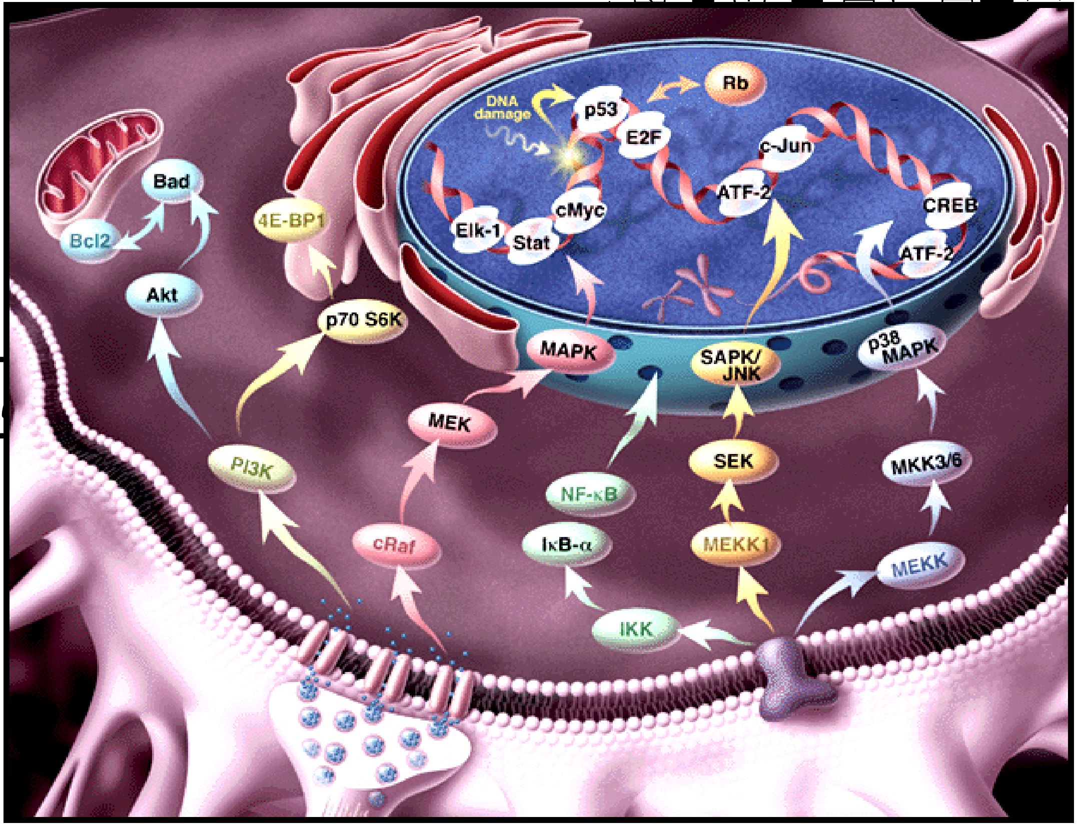
Yeast cell cycle

From flow cytometry data to signaling networks



Picture: John Albeck

Flow



K. Sachs, 2005

Outline

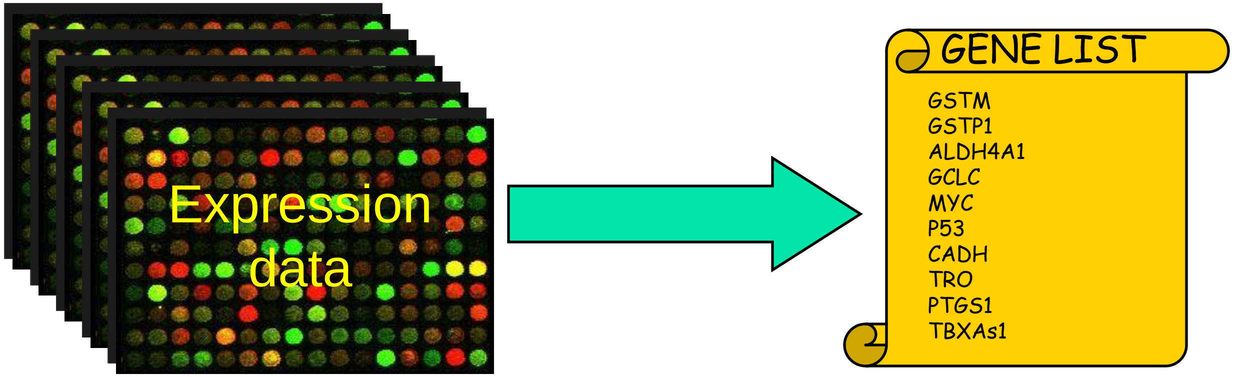
- The problem of learning regulatory, signaling and metabolic networks from data
- A quick intro to Bayesian networks
- Algorithms for learning Bayesian networks from data
- Examples
  - Glutathione metabolism from humans (expression data)
  - Regulatory network from yeast cell cycle (expression data)
  - T-cell signaling from humans (flow cytometry data)

Challenges

- The cell is a complex stochastic domain: signal transduction, metabolic and regulatory pathways all interconnected.
- Pathways are controlled by combination of many mechanisms.
- We only observe mRNA levels and/or protein levels.
- Measurements are noisy.

Some initial approaches

- Classification of expression data
  - Reveals genes that are differentially expressed.
  - Disadvantage: does not reveal structural relationships between genes.



Expression data

GENE LIST

GSTM
GSTP1
ALDH4A1
GCLC
MYC
P53
CADH
TRO
PTGS1
TBXAs1

Some initial approaches

- Clustering techniques
  - Many interesting clusters of co-regulated genes
  - No system-level insight.

Some initial approaches

- Boolean networks
  - Deterministic models of interactions between genes.
  - Disadvantage: deterministic. We need stochastic models for representing interactions.

Why Bayesian networks?

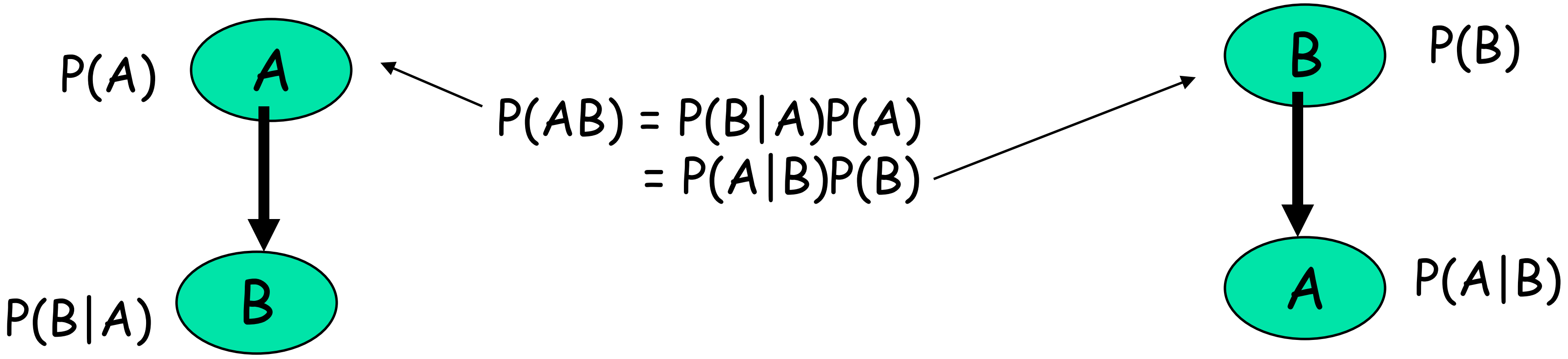
- The important science/technology to come out of AI in the last 15 years.
- Underlies all important applications today.
- Frames every question as the estimation of a conditional probability
  - P(disease/problem|set of symptoms)
  - P(email is spam|email text+header)
  - P(hurricane will hit place X|movement history)
  - P(sentence|acoustic signal)
  - P(regulatory network|gene exp data)

Bayesian networks: the model

- A Bayesian network B = (V,E) is a directed acyclic graph in which each node in V is annotated with quantitative probability information.
  - A set V of random variables are the nodes of the network. They can be continuous or discrete.
  - If there is an edge from node X to node Y in E, then X is said to be the parent of Y.
  - Each node X in V has a conditional probability distribution P(X|Parents(X)) associated with it.

An example

- A Bayesian network is a compact representation of the joint distribution over a set of random variables.
    - $P(X_1, X_2, \ldots, X_n)$



$$P(AB) = P(B|A)P(A) \\ = P(A|B)P(B)$$

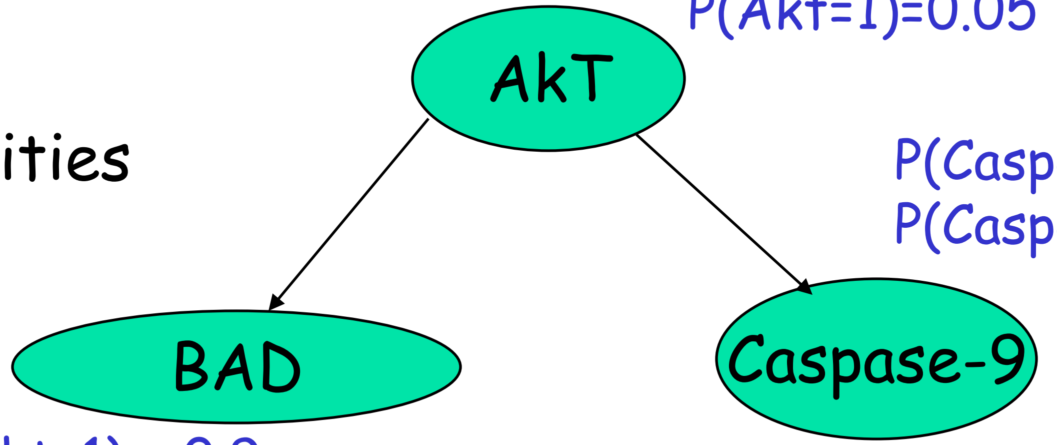
Example: Akt pathway

Random variables: Akt, BAD, caspase-9

Conditional independencies:

$P(\text{BAD and caspase-9}|\text{AKT}) = P(\text{BAD}|\text{Akt})P(\text{Caspase-9}|\text{AkT})$

2+2+1 probabilities



$P(\text{Akt}=1)=0.05$

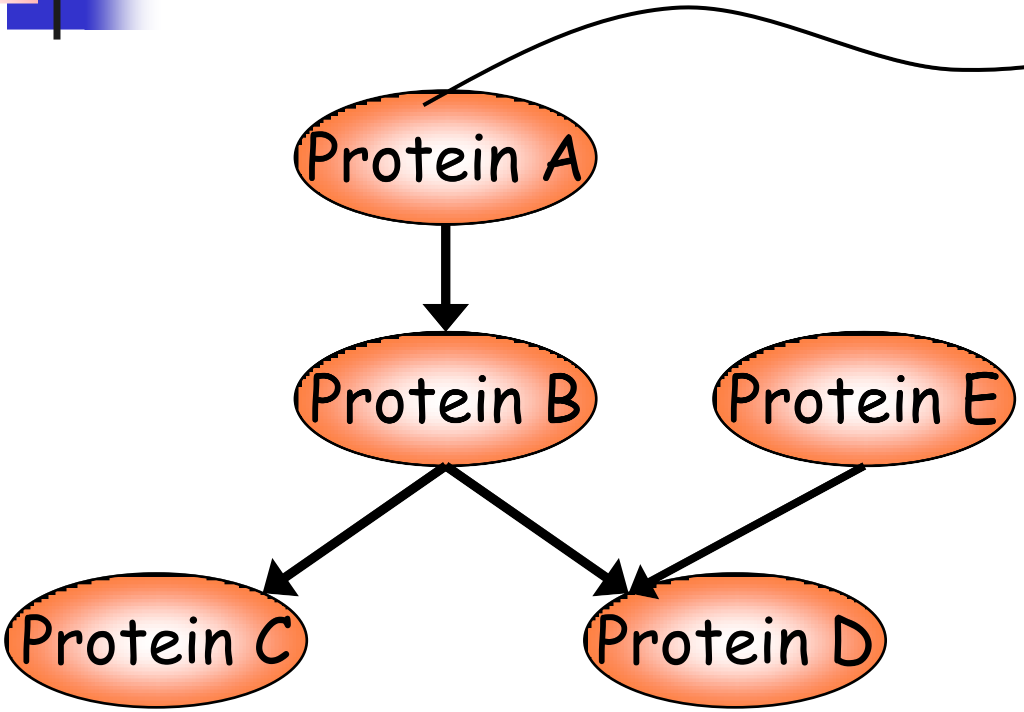
$P(\text{Caspase-9}=1|\text{Akt}=1)=0.1$
$P(\text{Caspase-9}=1|\text{Akt}=0)=0.9$

$P(\text{BAD}=1|\text{Akt}=1) = 0.9$
$P(\text{BAD}=1|\text{Akt}=0)= 0.1$

Akt pathway

- To specify full distribution, assuming that the three variables are discretized into high and low, we need $2^3-1=7$ probabilities.
- The Bayesian netwok representation needs 5 probabilities.
- In general, for an n variable problem, reduction of parameters from 2^n to $n*2^k$, if every node has k parents ($k<<n$).

Another example

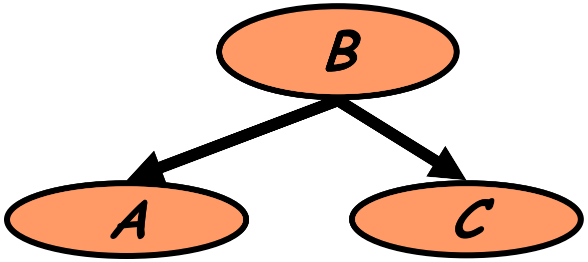


If Protein A is low(0), Protein B is high(1) with probability 0.8

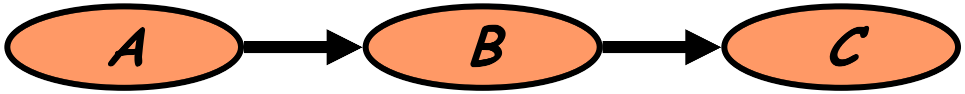
$$P(B=1|A=0) = 0.8$$
$$P(B=1|A=1) = 0.3$$

Adapted from Sachs, 2005

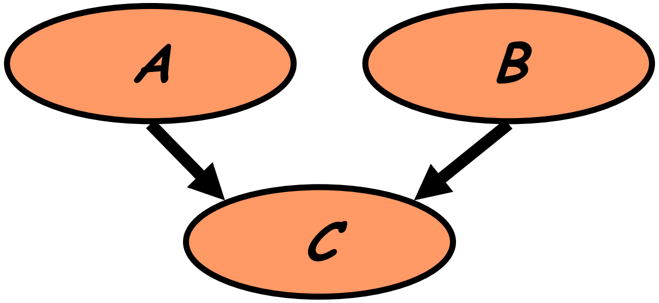
Summary of dependency types



Common cause



Intermediate gene

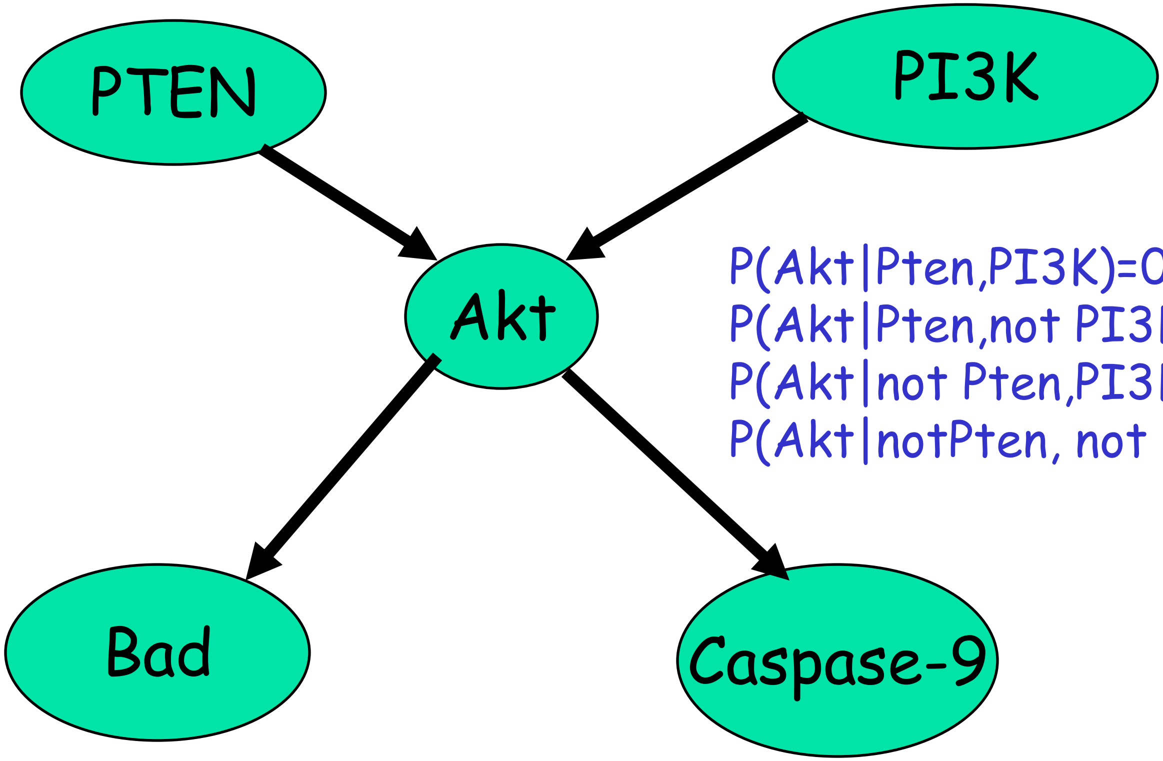


Common effects

A simple Bayesian network

P(Pten=1)=0.8

P(PI3K=1)=0.7



P(Akt|Pten,PI3K)=0.95
P(Akt|Pten,not PI3K)=0.95
P(Akt|not Pten,PI3K)=0.29
P(Akt|notPten, not PI3K)=0.001

P(BAD=1|Akt=1) = 0.9
P(BAD=1|Akt=0)= 0.05

P(Caspase-9=1|Akt=1)=0.2
P(Caspase-9=1|Akt=0)=0.9

Conditional independence

- The topology of the network reflects a set of conditional independence statements.
  - PTEN and PI3K directly affect the probability of the Akt levels being high, but whether or not Bad or Caspase-9 is high depends on the Akt levels alone. Bad and Caspase-9 do not directly respond to PTEN and PI3K levels, the interaction is mediated only through Akt.
  - Bad level is conditionally independent of Caspase-9 level given Akt level.

Computing joint probability distributions

- Any entry in the joint probability distribution can be calculated from the Bayesian network.

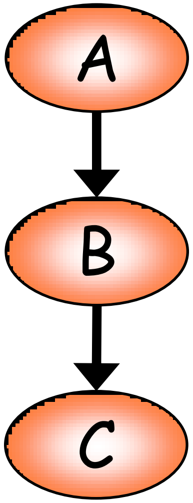
Computing joint probabilities

$$P(X_1 = x_1, \ldots, X_n = x_n) = \prod_{i=1}^{n} P(X_i = x_i \mid Parents(X_i))$$

Learning Bayesian Models

- Using data D, find the Bayesian network G that is most likely given the data, i.e. G that maximizes P(G|D).
- Graph structure is known; the conditional probability distributions are unknown.
  - Recovering optimal conditional probability distributions when the graph is known is "easy".
- Graph structure and the conditional probability distributions are unknown.
  - Recovering optimal graph structure is NP-hard.

Learning CPTs



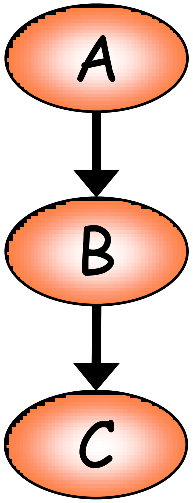
Known structure!

A	B	C
On	On	On
On	Off	Off
On	On	Off
On	On	On
On	On	On
On	On	On
Off	Off	Off
Off	On	On
Off	Off	Off
Off	Off	Off
Off	Off	Off

From Sachs 2005

Learning CPTs

P(B='On'|A='On') = 0.83

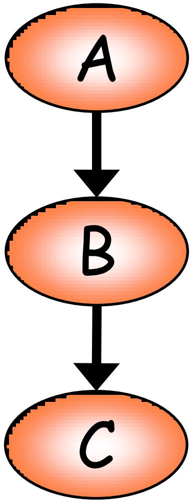


5/6 = 0.83

A	B	C
On	On	On
On	Off	Off
On	On	Off
On	On	On
On	On	On
On	On	On
Off	Off	Off
Off	On	On
Off	Off	Off
Off	Off	Off
Off	Off	Off

From Sachs 2005

Learning CPTs



P(B='On'|A='On') = 0.83

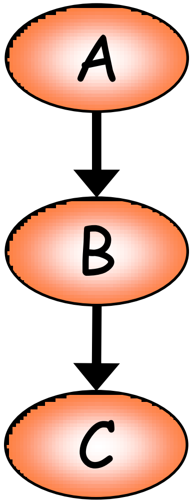
P(B='Off'|A='Off') = 0.8

4/5 = 0.8

A	B	C
On	On	On
On	Off	Off
On	On	Off
On	On	On
On	On	On
On	On	On
Off	Off	Off
Off	On	On
Off	Off	Off
Off	Off	Off
Off	Off	Off

From Sachs 2005

Learning CPTs



P(B='On'|A='On') = 0.83

P(B='Off'|A='Off') = 0.8

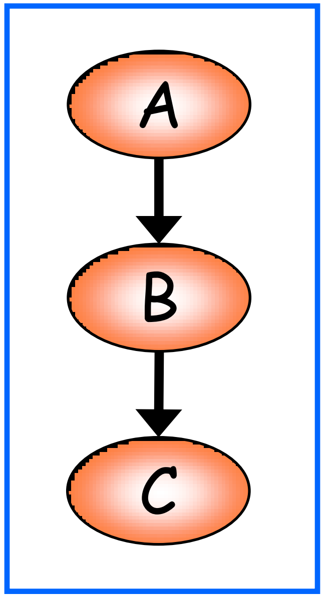
P(C='On'|A='On') = 0.66

4/6 = 0.66

A	B	C
On	On	On
On	Off	Off
On	On	Off
On	On	On
On	On	On
On	On	On
Off	Off	Off
Off	On	On
Off	Off	Off
Off	Off	Off
Off	Off	Off

From Sachs 2005

Learning CPTs



P(B='On'|A='On') = 0.83

P(B='Off'|A='Off') = 0.8

P(C='On'|A='On') = 0.66

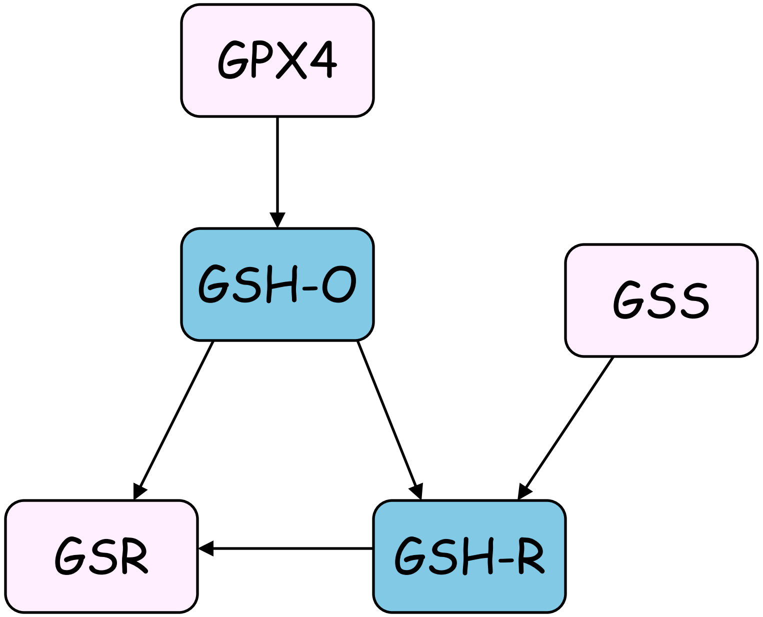
P(C='On'|B='On') = 0.8

4/5 = 0.8

A	B	C
On	On	On
On	Off	Off
On	On	Off
On	On	On
On	On	On
On	On	On
Off	Off	Off
Off	On	On
Off	Off	Off
Off	Off	Off
Off	Off	Off

From Sachs 2005

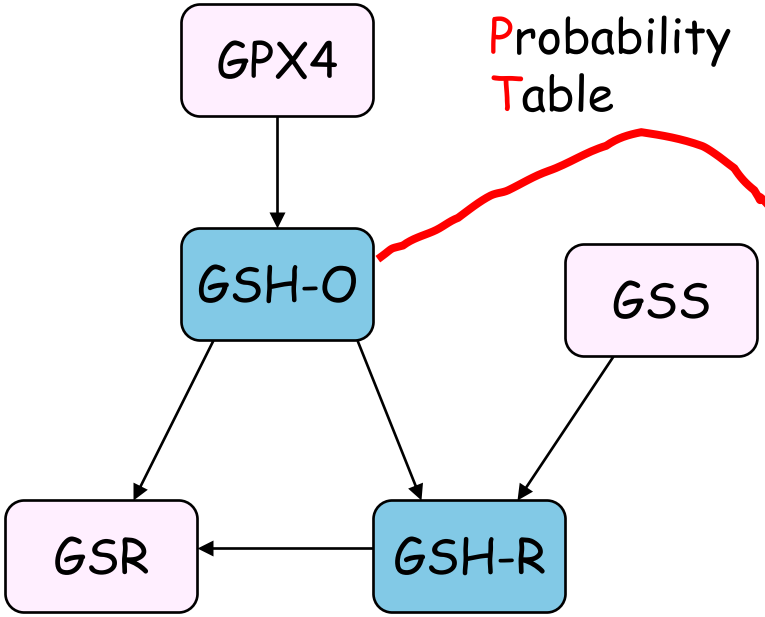
Modeling cellular processes: topology of glutathione network



A portion of the GSH network

- Three alternate synthesis pathways for GSH-R: from GSH-O by GSR, from GSH-O by GPX4, and independently from GSS.
- Edges here are not causal; edge directions chosen to
  - Keep network acyclic
  - Make nodes have no more than two to three parents.
- Network is an alternate but correct factoring of the full joint distribution on expression levels.

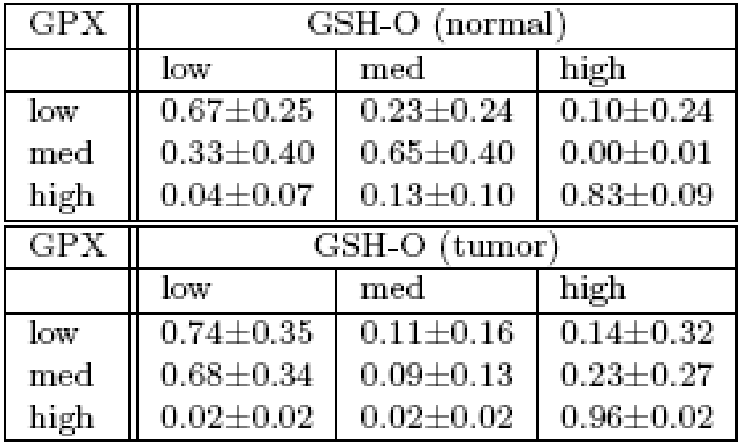
Modeling cellular processes: the quantitative parameters



A portion of the GSH network

Conditional
Probability
Table

- Our models have a quantitative component. Each node has a conditional probability distribution associated with it.
- These models are learned from data!



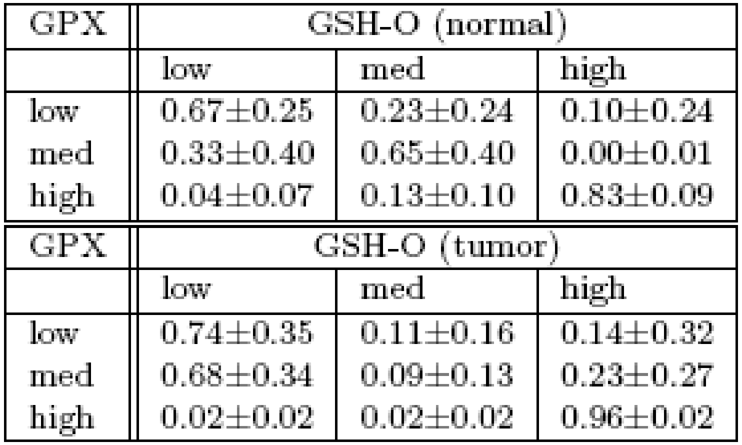
GPX	GSH-O (normal)		
	low	med	high
low	0.67±0.25	0.23±0.24	0.10±0.24
med	0.33±0.40	0.65±0.40	0.00±0.01
high	0.04±0.07	0.13±0.10	0.83±0.09

GPX	GSH-O (tumor)		
	low	med	high
low	0.74±0.35	0.11±0.16	0.14±0.32
med	0.68±0.34	0.09±0.13	0.23±0.27
high	0.02±0.02	0.02±0.02	0.96±0.02

Learning CPTs from data

- To learn a CPT of the form P(Y|X), where Y and X are both observed, we can use maximum likelihood estimation.
    - P(Y|X)=count(X&Y)/count(Y)
- When there are unobserved variables, we use the expectation maximization (EM) procedure to make the best guess for the values of the unobserved variables given the observed ones, and readjust the parameters of the network based on the guesses. We find the most likely network parameters given the observed data.

Component network learning



GPX	GSH-O (normal)		
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med	0.33±0.40	0.65±0.40	0.00±0.01
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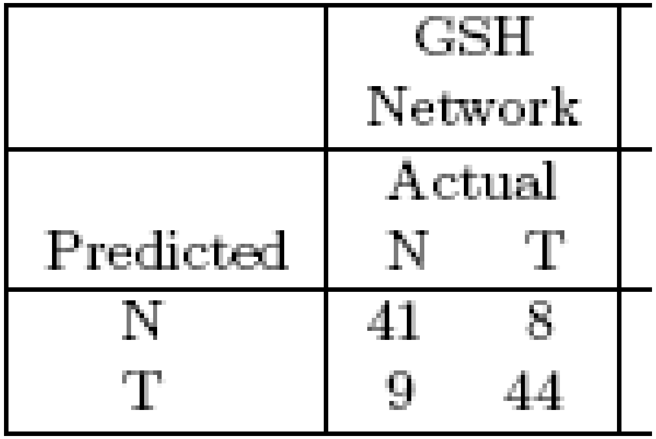
GPX	GSH-O (tumor)		
	low	med	high
low	0.74±0.35	0.11±0.16	0.14±0.32
med	0.68±0.34	0.09±0.13	0.23±0.27
high	0.02±0.02	0.02±0.02	0.96±0.02

Note that tumor cells produce lower than normal amounts of GSH-O when GPX levels are medium.

- We learn **separate network** parameters for normal cells and diseased cells for each metabolic process we model.
- Differences in parameters indicate differences in the underlying process.

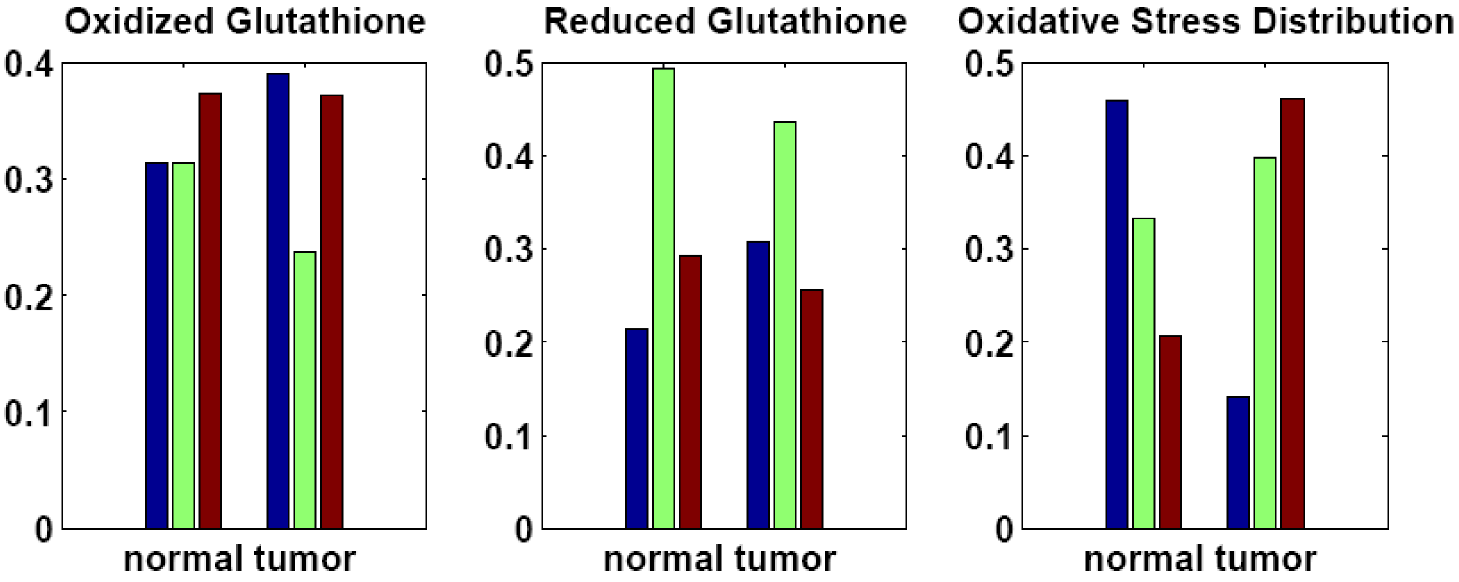
Robustness of EM learning

Leave-one-out Cross validation results for the GSH network



	GSH Network	
	Actual	
Predicted	N	T
N	41	8
T	9	44

Predictions from GSH network



We can make predictions about metabolite levels from the two learned networks. It is remarkable that we can predict that the level of oxidative stress in tumor cells is much higher in tumor cells using networks learned from the gene expression data alone!

Learning network structure

- Find the network structure that has maximum likelihood with respect to the data
    - Find G that maximizes P(G|D).

The Bayesian approach

Network Posterior

Marginal Likelihood

$$P(G \mid D) \propto P(D \mid G) P(G)$$

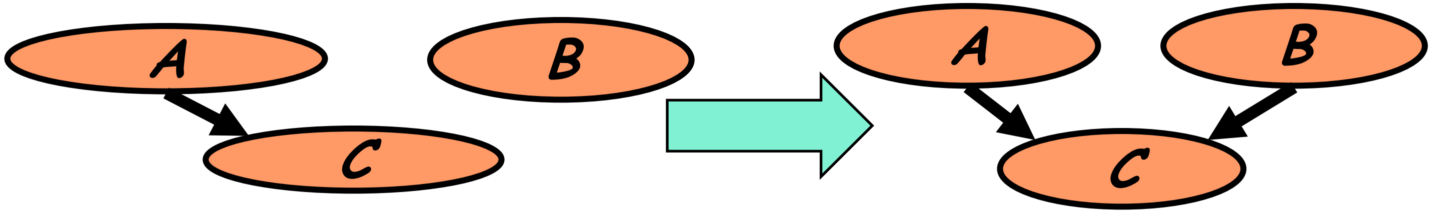
Prior over Networks

Key idea: Use *P(G/D)* to evaluate a network given a particular data set.

Learning network structure

- The structure (G) learning problem is NP-hard => heuristic search for best model must be applied, generally bring out a **locally** optimal network.
- It turns out, that richer structures give higher likelihood P(D|G) to the data (adding an edge to the graph is always preferable).

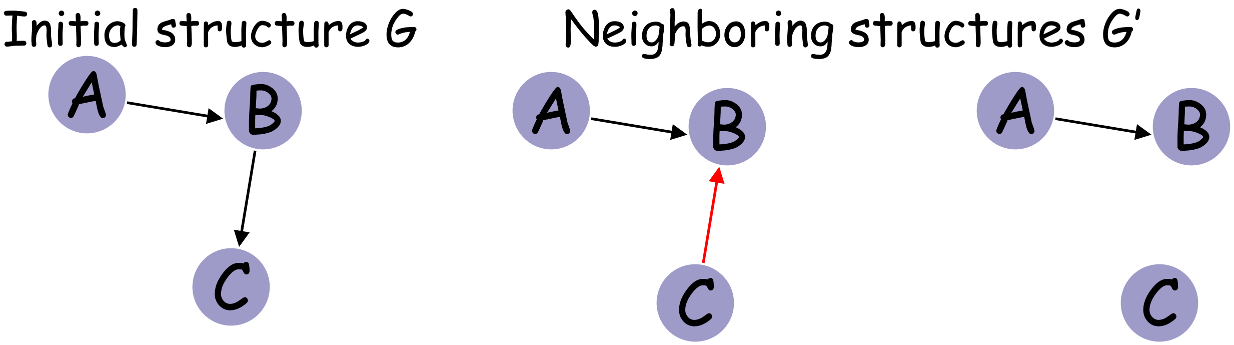
Learning structure



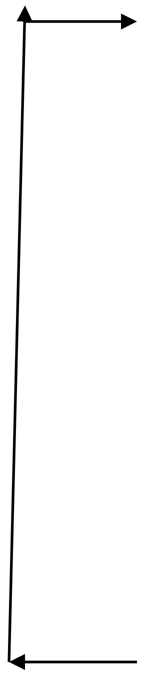
- If we add B to Parents(C) , we have more parameters to fit → more freedom →
- But we prefer *simpler* (more explanatory) networks (Occam's razor!)
- Therefore, **practical** scores of Bayesian Networks compensate for the likelihood improvement by imposing a penalty on complex networks.

Local search

We change one edge and evaluate the gains made by this change



Search algorithm recipe



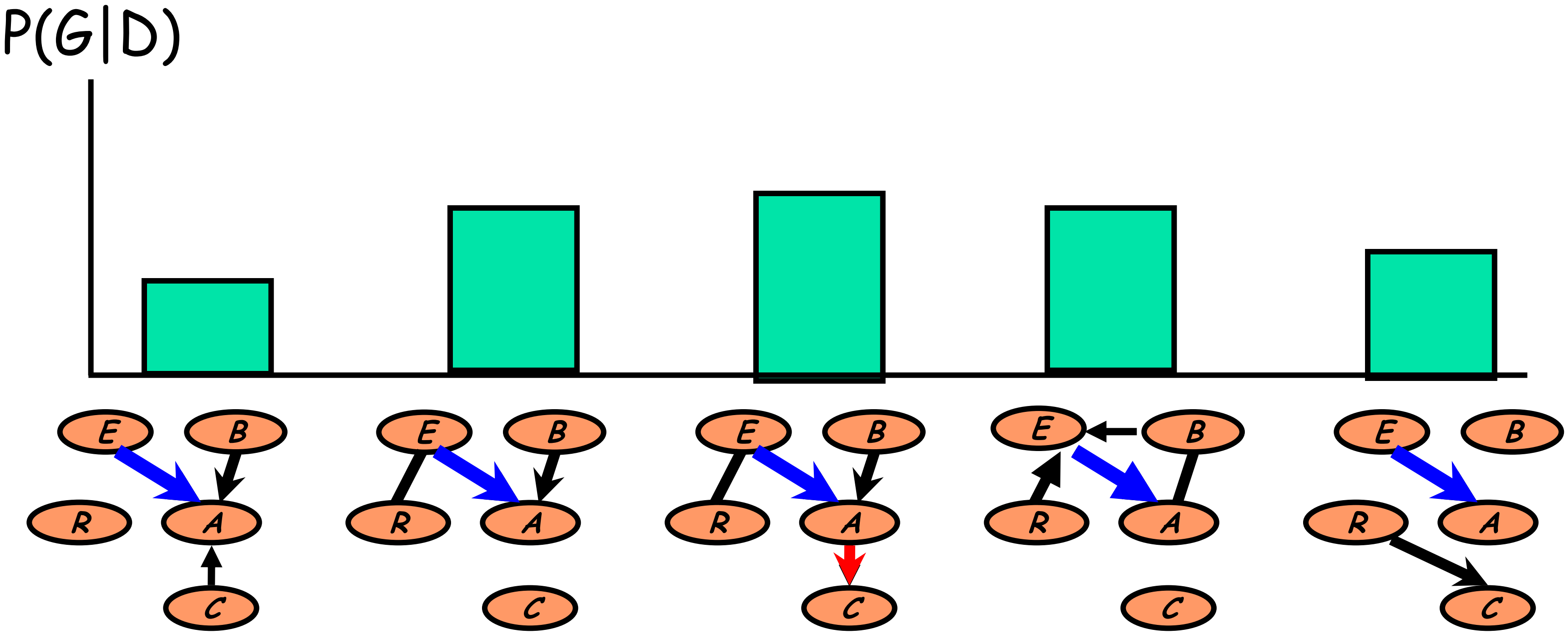
- Start with a random graph G. Evaluate its likelihood wrt D, P(G|D).
- Until little improvement in likelihood
  - Perturb structure G by adding, deleting or reversing edge
  - Accept change if likelihood improves.
- End

Randomized restarts

Difficulty #1

- We do not have enough data to uniquely identify a high-scoring network.
    - Exponentially many networks with the same P(G|data) score!
- Solution: generate many high-scoring networks and extract common features.

Evaluating networks



Look for features **common to many models**

Difficulty #2

- What space of graph perturbations to consider?
- Solution: sparse candidate algorithm (Friedman 1999)
  - Limit potential parents to k most correlated variables.

Estimating statistical confidence in features

- To what extent does the data support a given feature?
- An effective and relatively simple approach for estimating confidence is the bootstrap method.

The bootstrap method

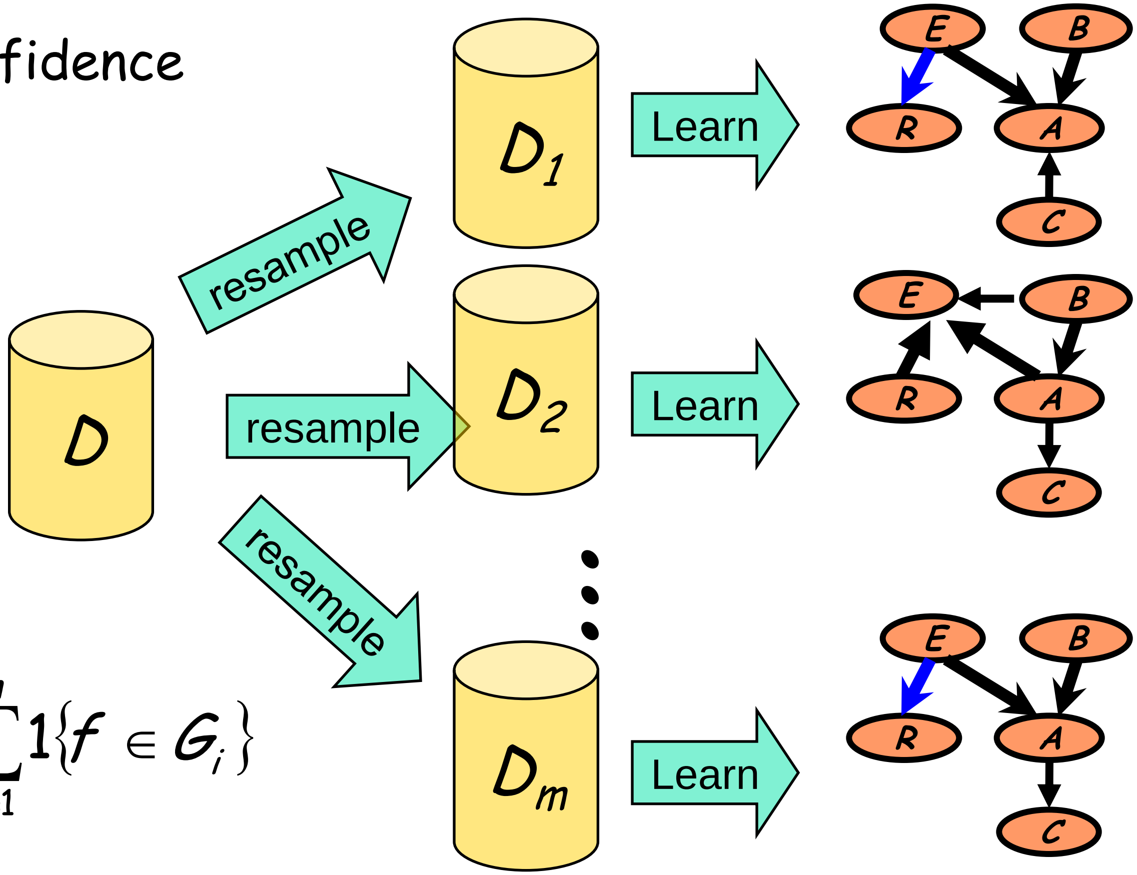
- For *i* = 1, …, *m*
  - Re-sample with replacement *N* instances from *D*. Denote by D_i the resulting dataset.
  - Apply the learning procedure on D_i to induce a network structure *G*.
- For each feature *f* of interest calculate

$$\text{conf}(f) = {}^{1}\!/_{m} \sum_{i=1}^{m} f(G_i)$$

  - where *f*(*G*) is 1 if *f* is a feature in *G*, and 0 otherwise.

Bootstrap illustrated

C(f) is the confidence in a feature.



$$C(f) = \frac{1}{m}\sum_{i=1}^{m} 1\{f \in G_i\}$$

(c) Devika Subramanian, 2009

Improving statistical significance

Sparse Data

- Small number of samples
- "Flat posterior" -- many networks fit the data.

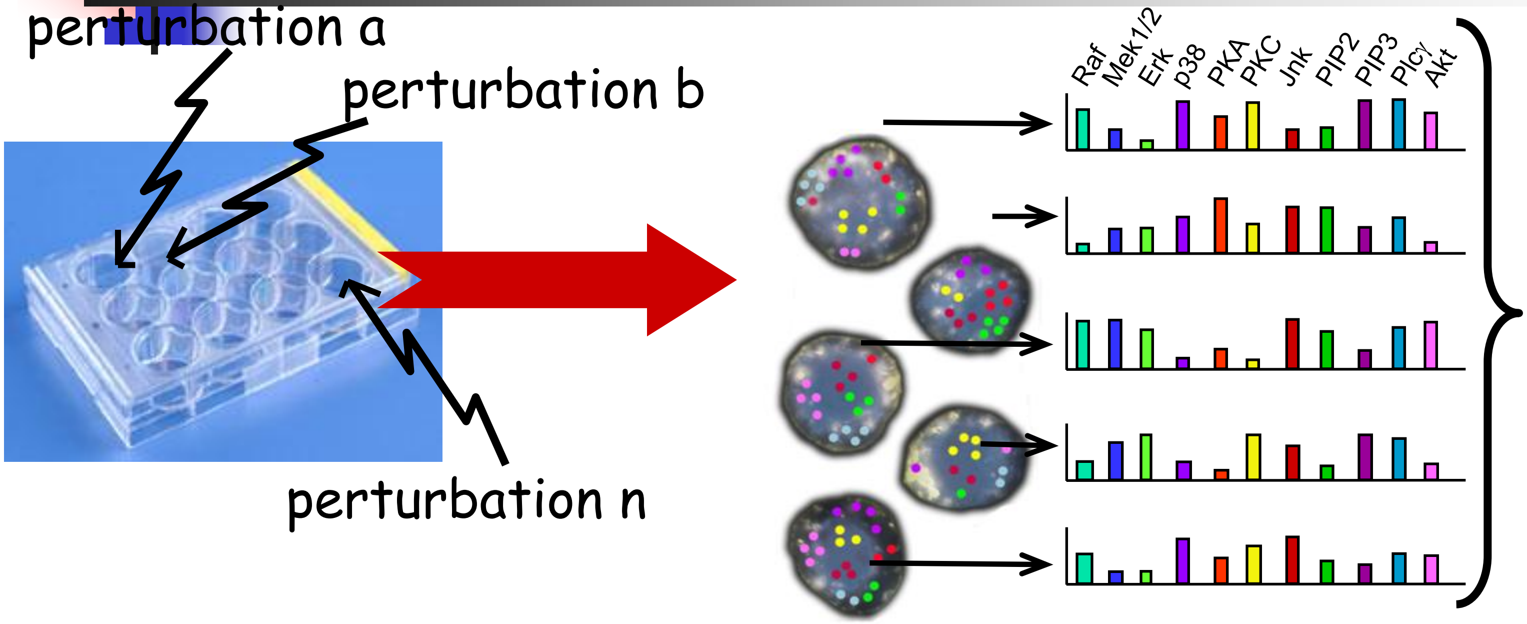
Solution

- estimate confidence in network **features**
- E.g., two types of features
  - **Markov** neighbors: *X* **directly** interacts with *Y* *(have mutual edge or a mutual child)*
  - **Order** relations: *X* is an **ancestor** of *Y*

T-Lymphocyte Data (Sachs 2005)

Conditions (96 well format)

12 Color Flow Cytometry



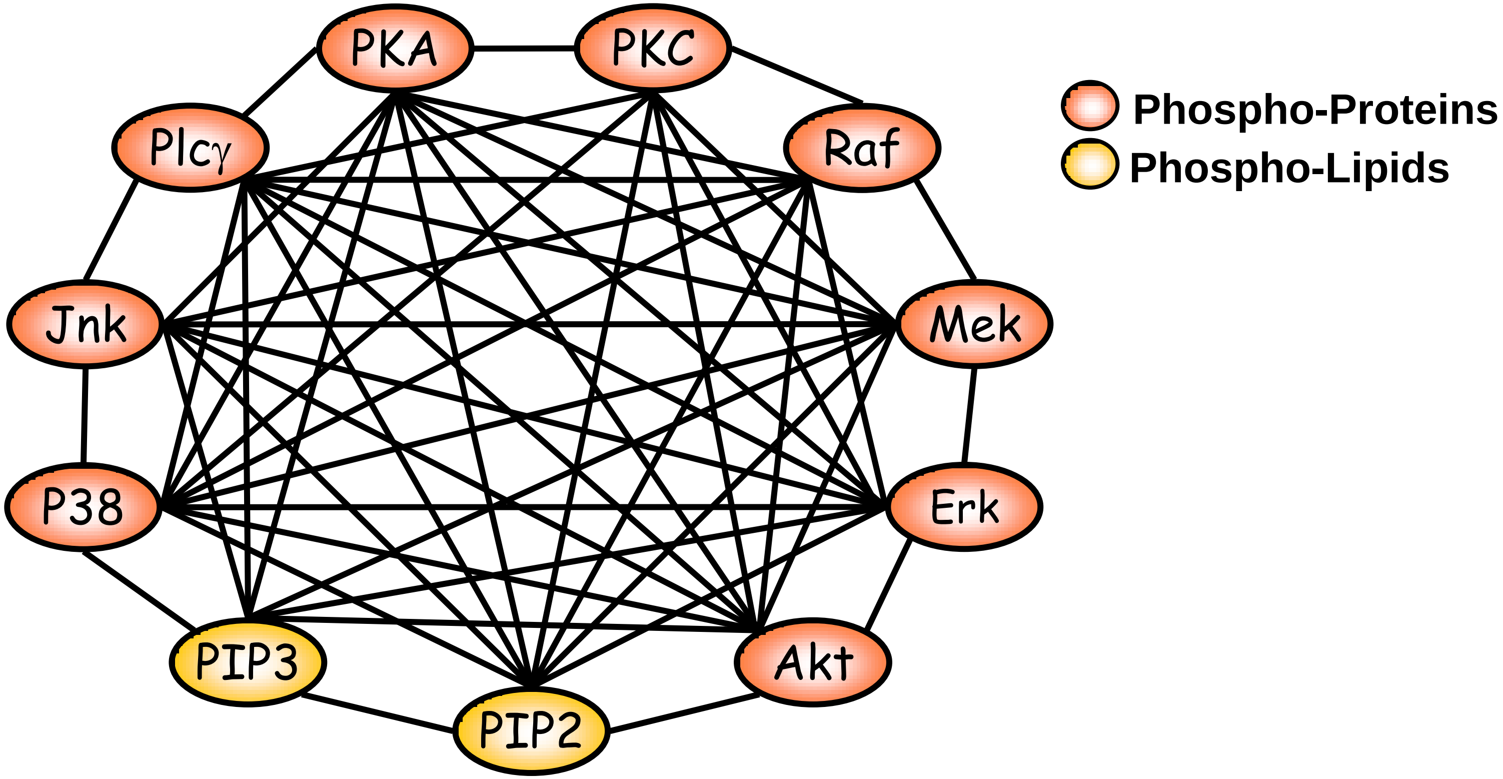
Datasets of cells
- *condition 'a'*
- *condition 'b'*
- *condition… 'n'*

- Primary human T-Cells
- 9 conditions
  - (6 **Specific** interventions)

- 9 phosphoproteins, 2 phospolipids
- 600 cells per condition
  - 5400 data-points

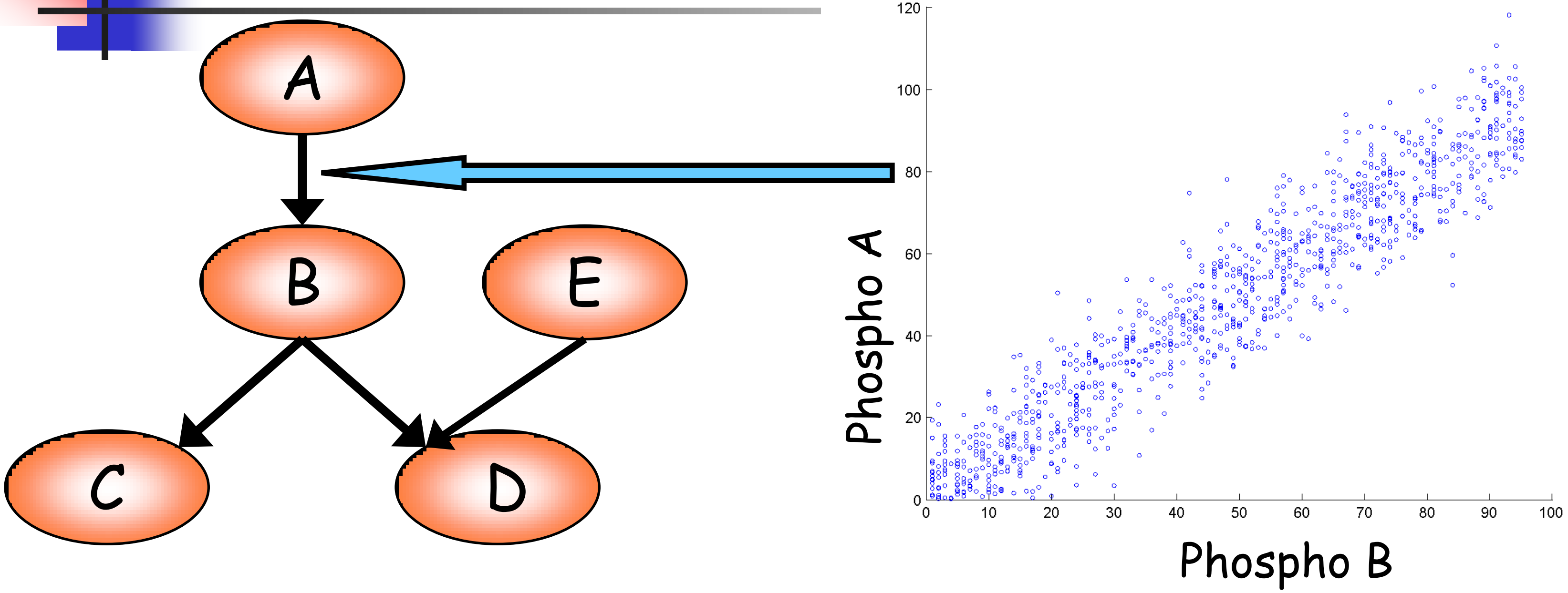
From Sachs 2005

Using correlations



From Sachs 2005

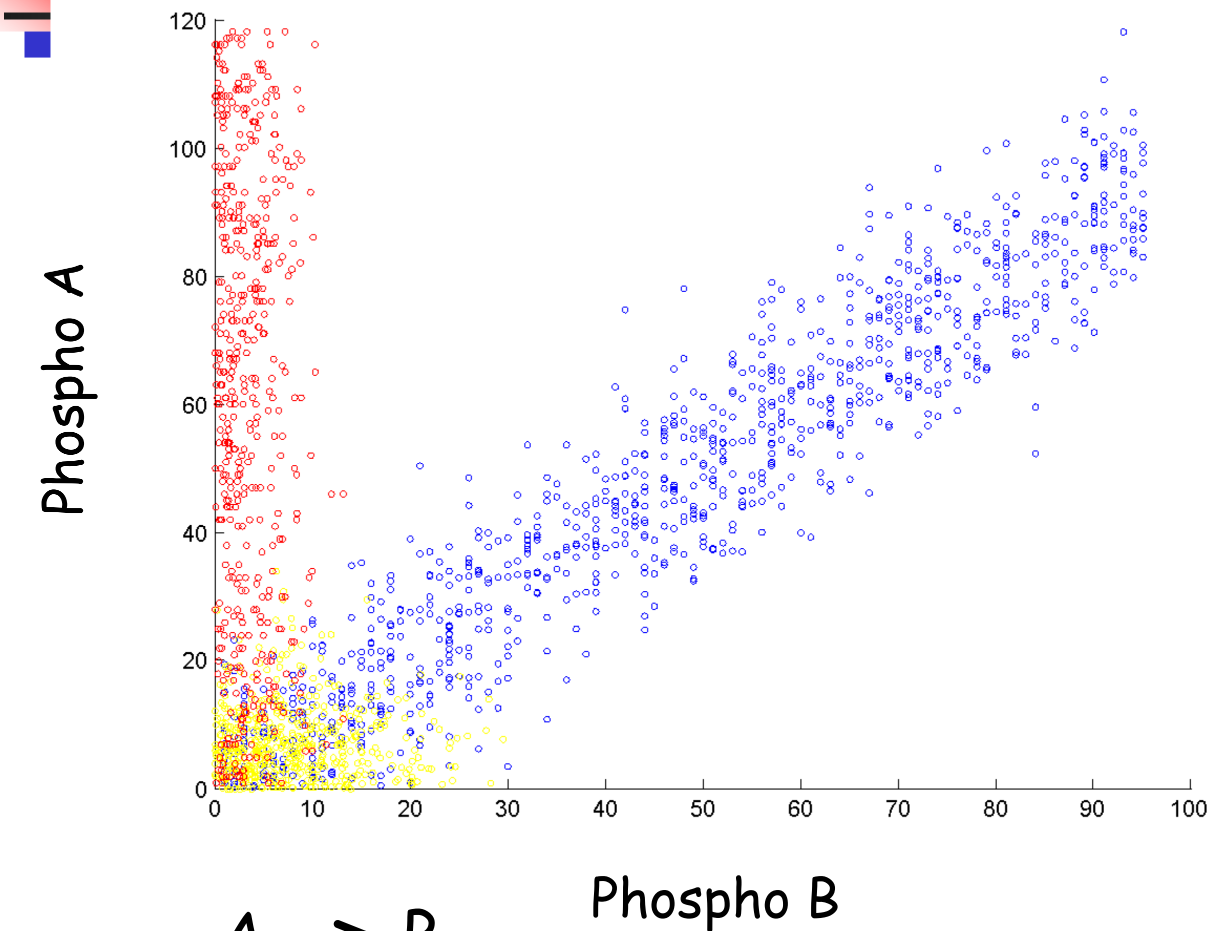
Statistical Dependencies



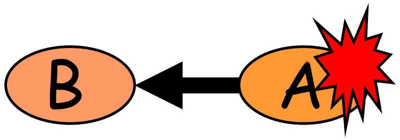
But, how can statistical dependencies determine directionality?

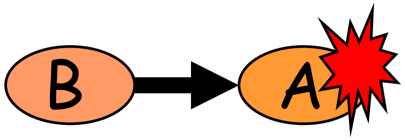
Sachs 2005

The Power of Interventions



- ● No Manipulations
- ● A inhibited
- ● B inhibited

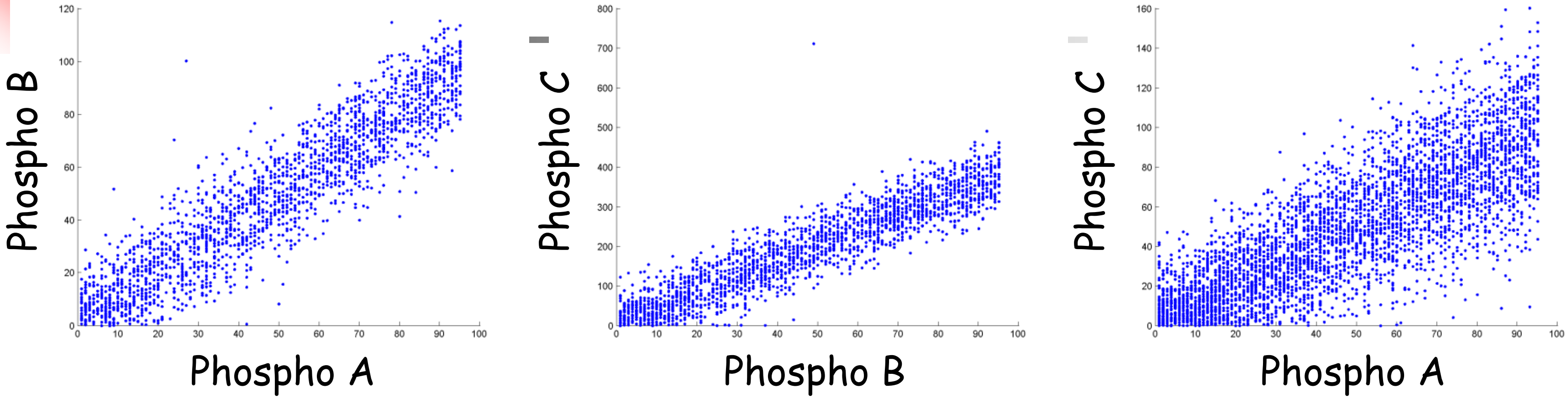


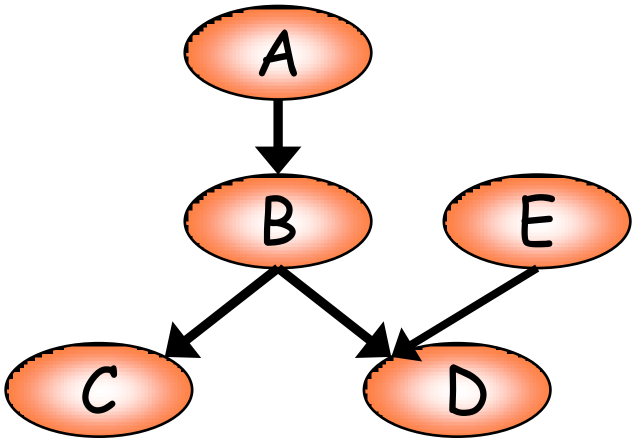


A → B

From Sachs 2005

Dismissing Edges

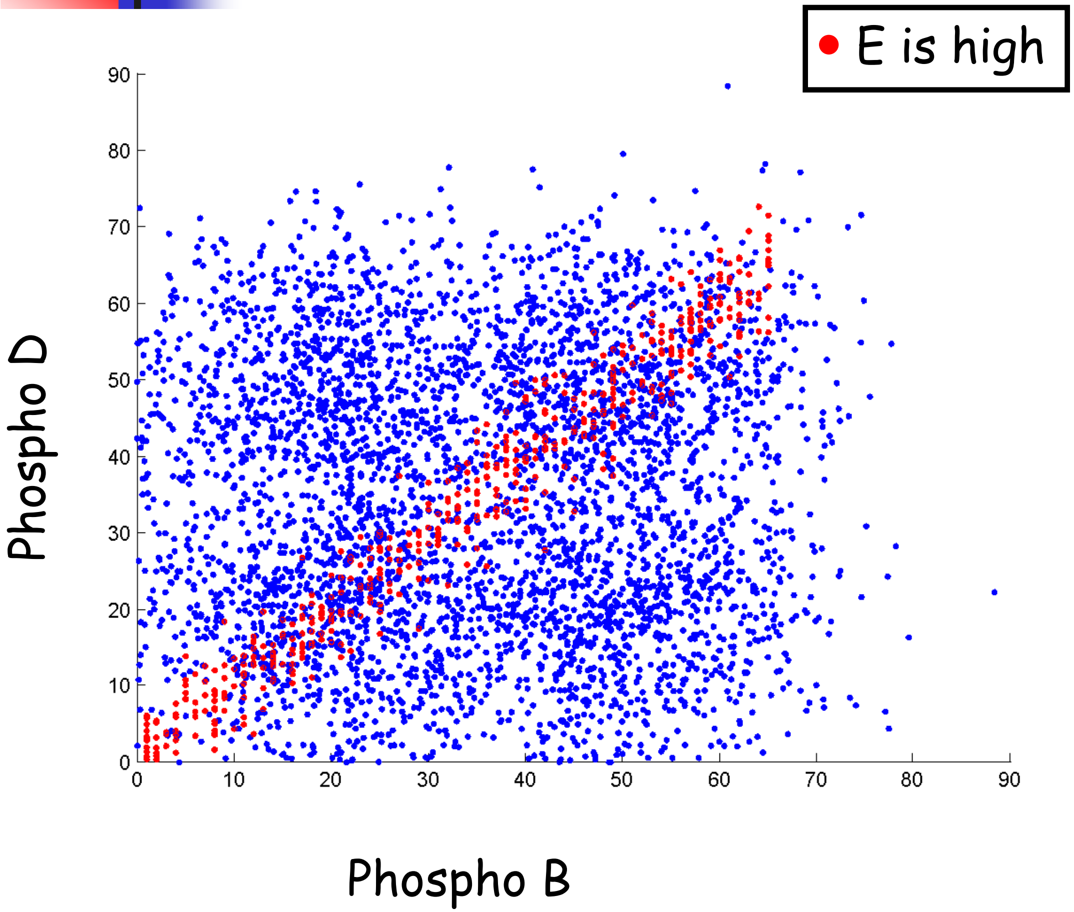




Edges A->B and B->C explain dependence of A and C dismissing the edge between them

Sachs 2005

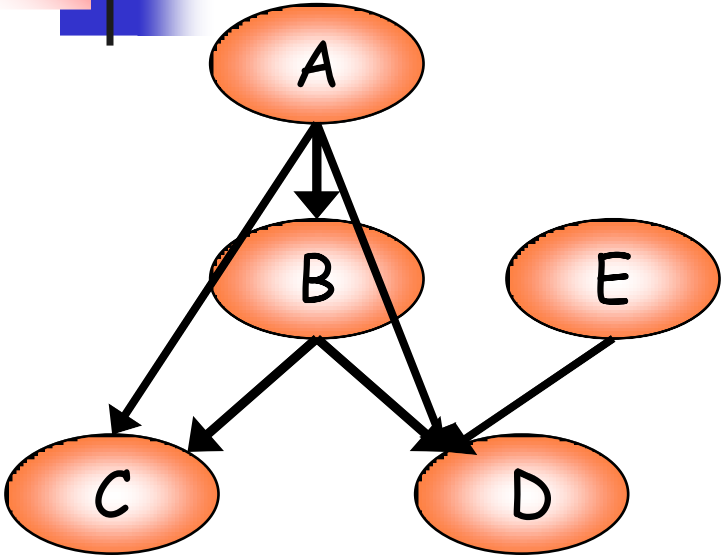
Context Specificity

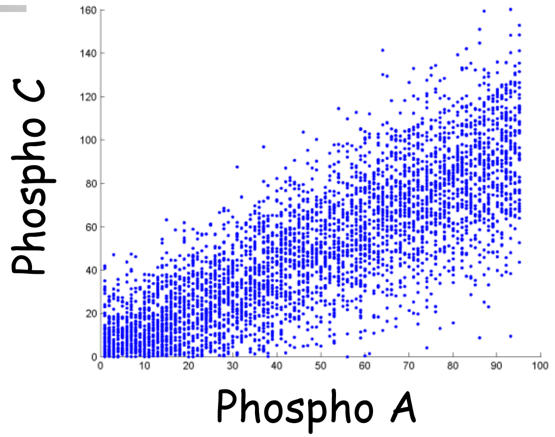


- B and D seem unrelated
- Relationship is revealed by considering simultaneous measurement of E
- Demonstrates the need for simultaneous measurements of variables
- Pairwise computational analysis (e.g. correlations) insufficient

Sachs 2005

Indirect Edges





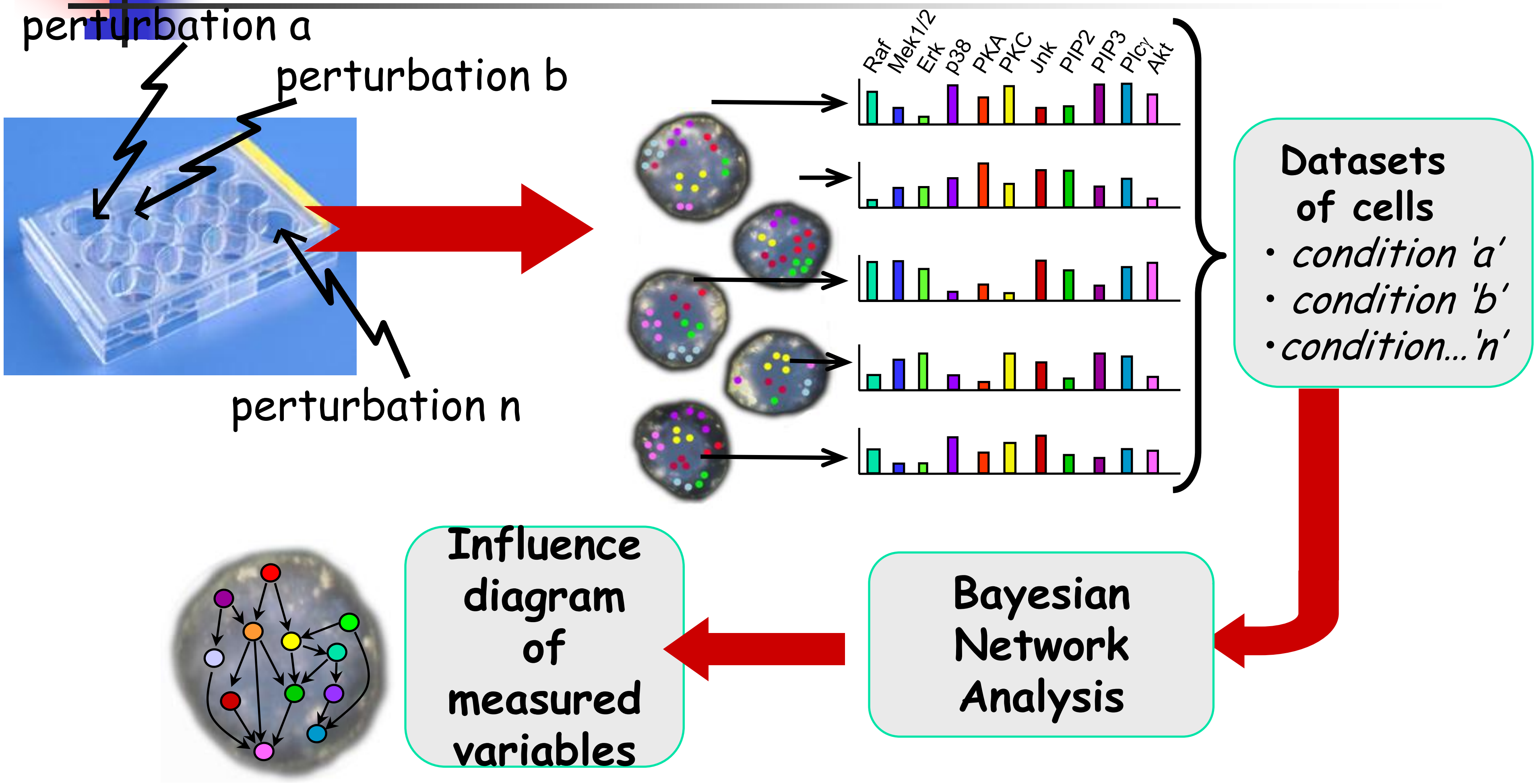
What would happen if B was not measured?

Sachs 2005

Summary

Conditions (96 well format)

Multiparameter Flow Cytometry



Datasets of cells

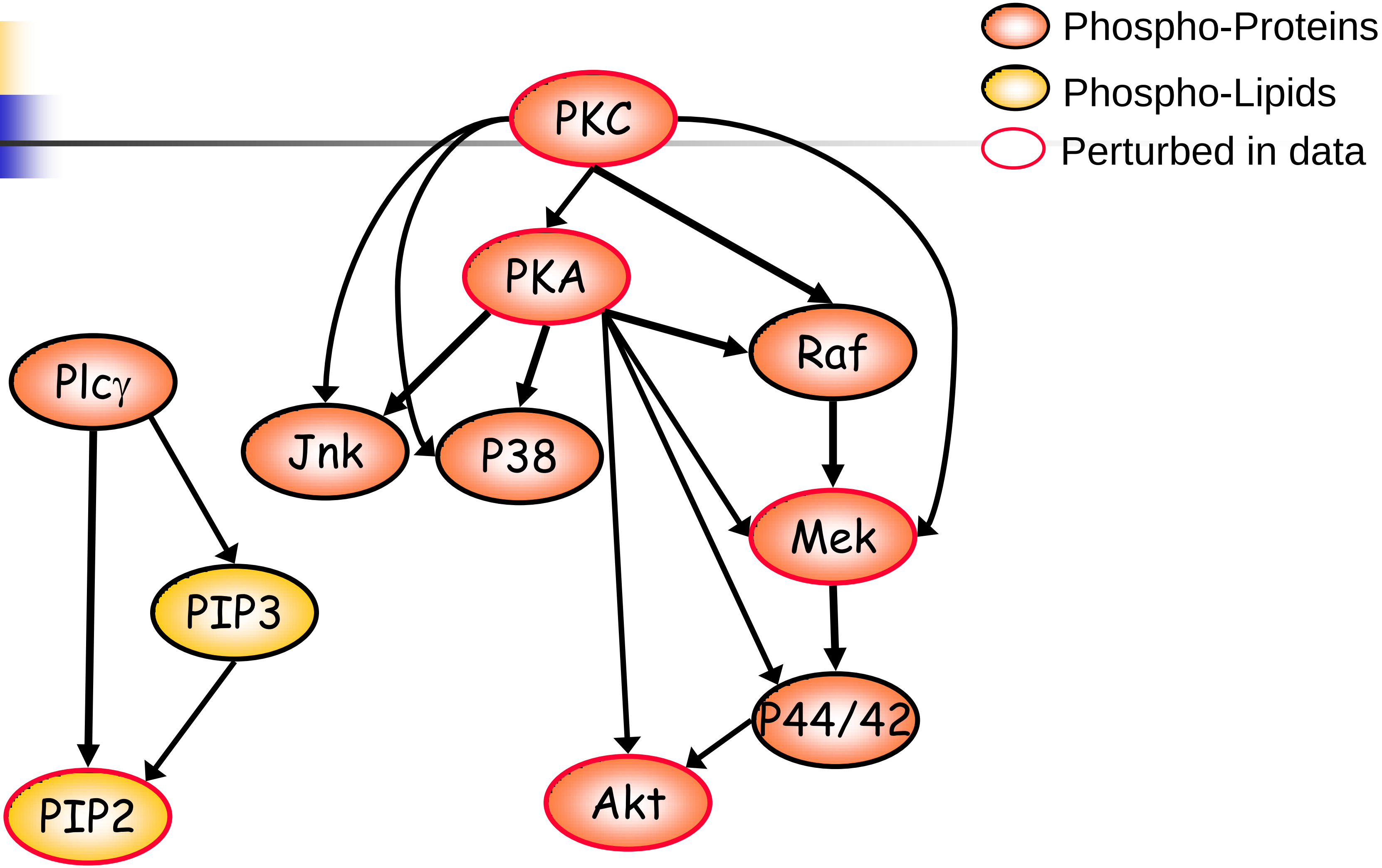
- *condition 'a'*
- *condition 'b'*
- *condition…'n'*

Bayesian Network Analysis

Influence diagram of measured variables

Sachs 2005

Inferred Network



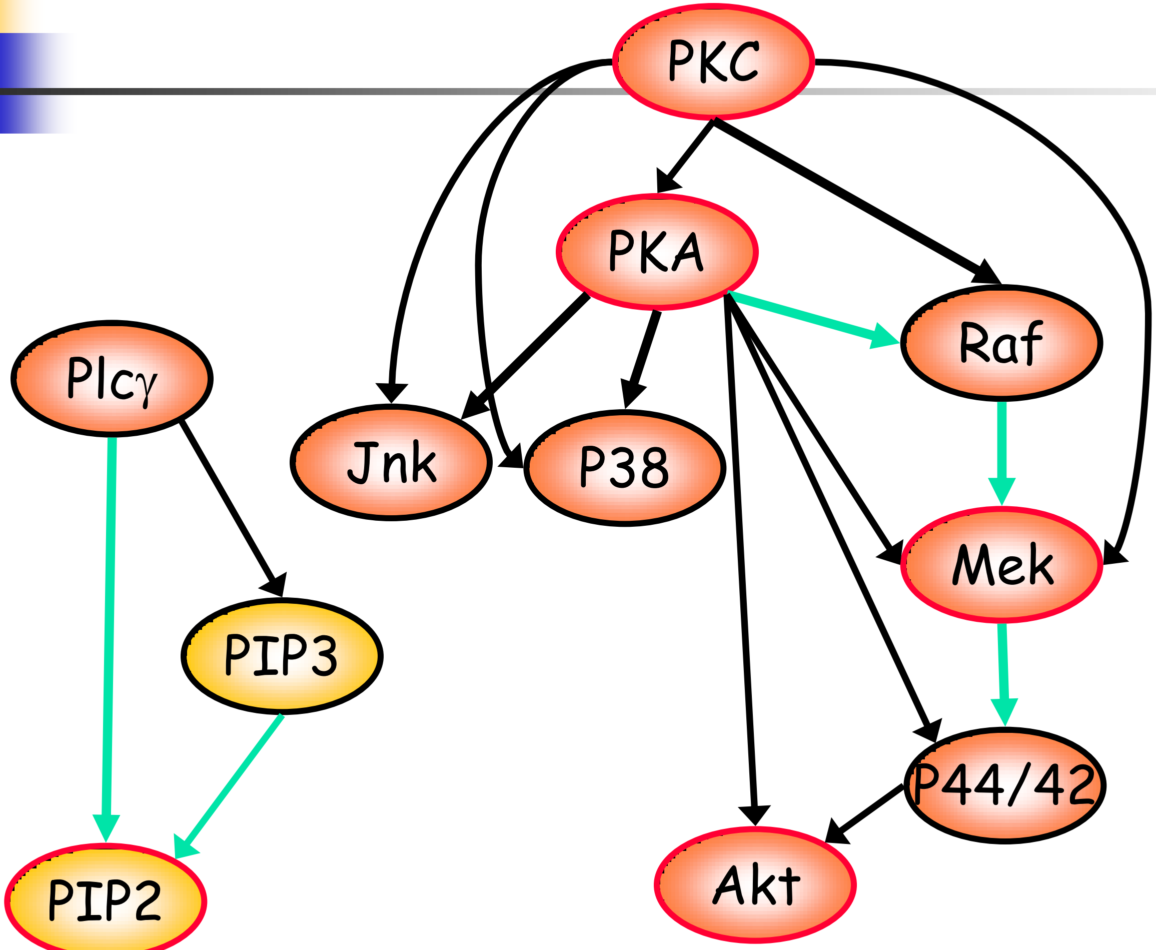
Sachs 2005

How good is the learned network?

Phospho-Proteins

Phospho-Lipids

Perturbed in data

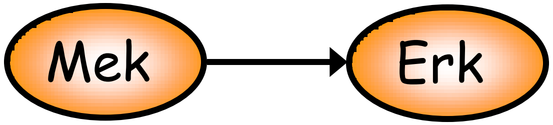


Direct phosphorylation

Sachs 2005

The need for cytometry data

- Direct phosphorylation:



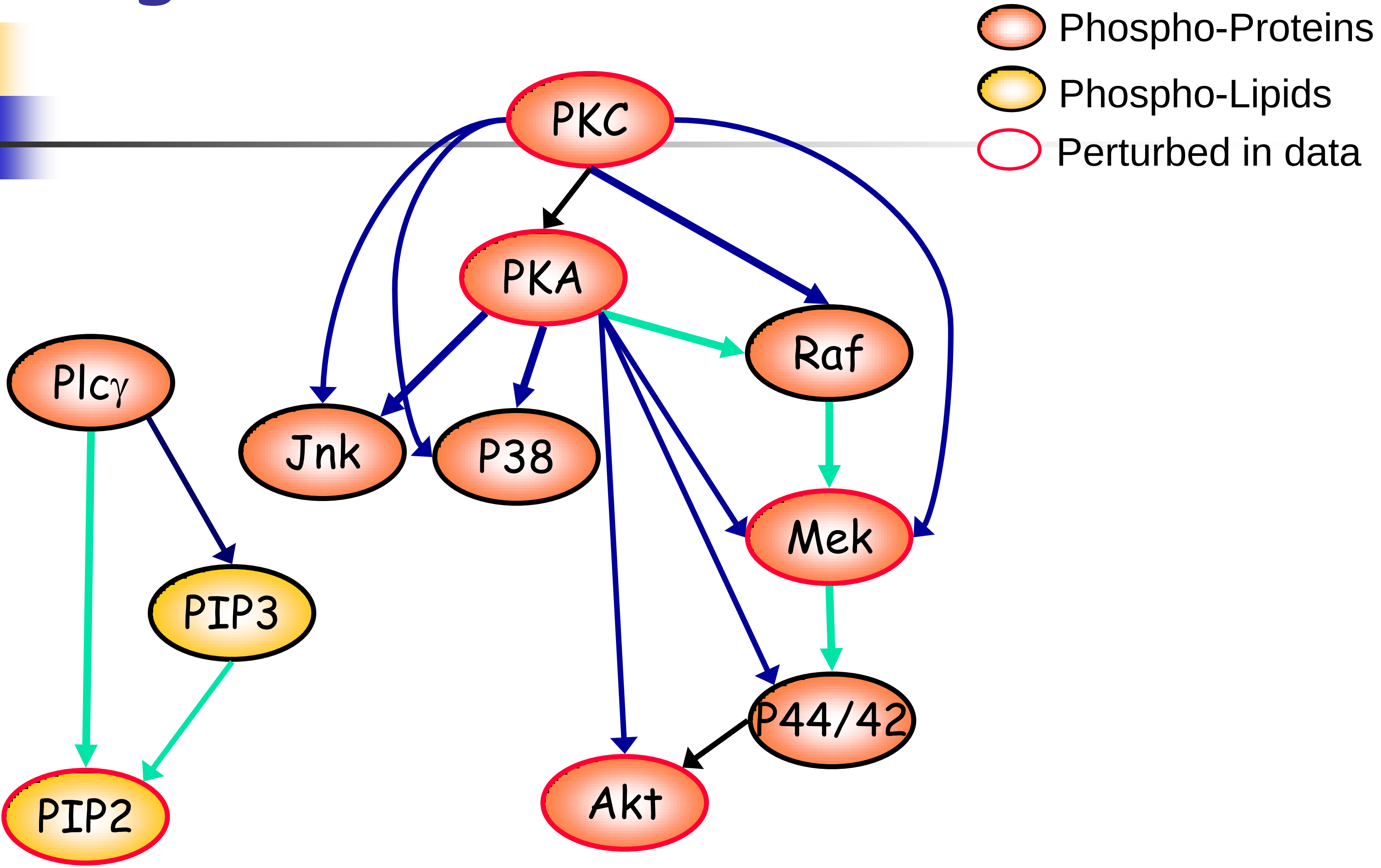
Difficult to detect using other forms of high-throughput data:

-Protein-protein interaction data

-Microarrays

Sachs 2005

How good is the learned network?



Indirect Signaling

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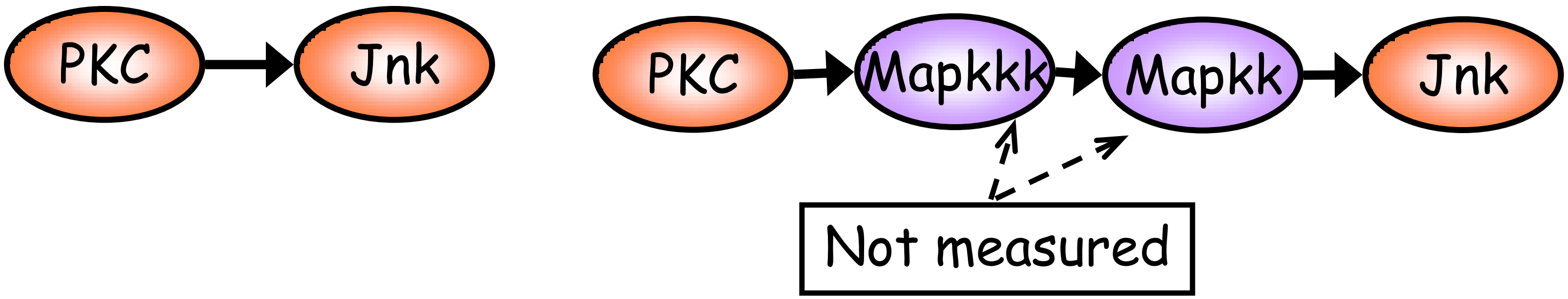
Ability to handle missing nodes

- Indirect signaling

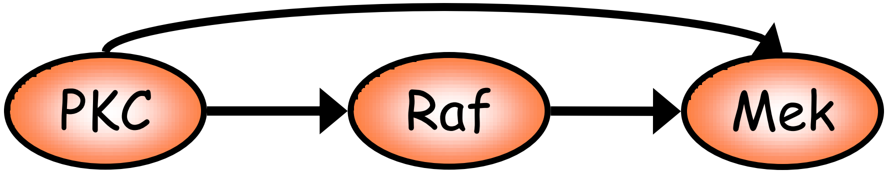


Indirect connections can be found even when the intermediate molecule(s) are not measured

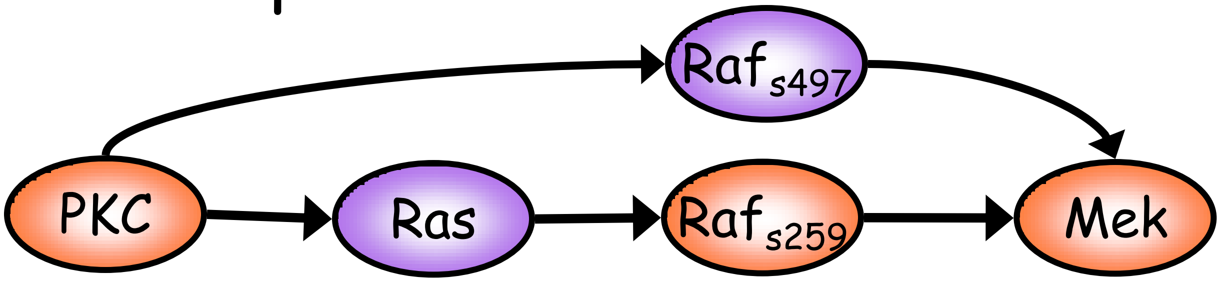
Sachs 2005

Indirect signaling

- Is this a mistake?

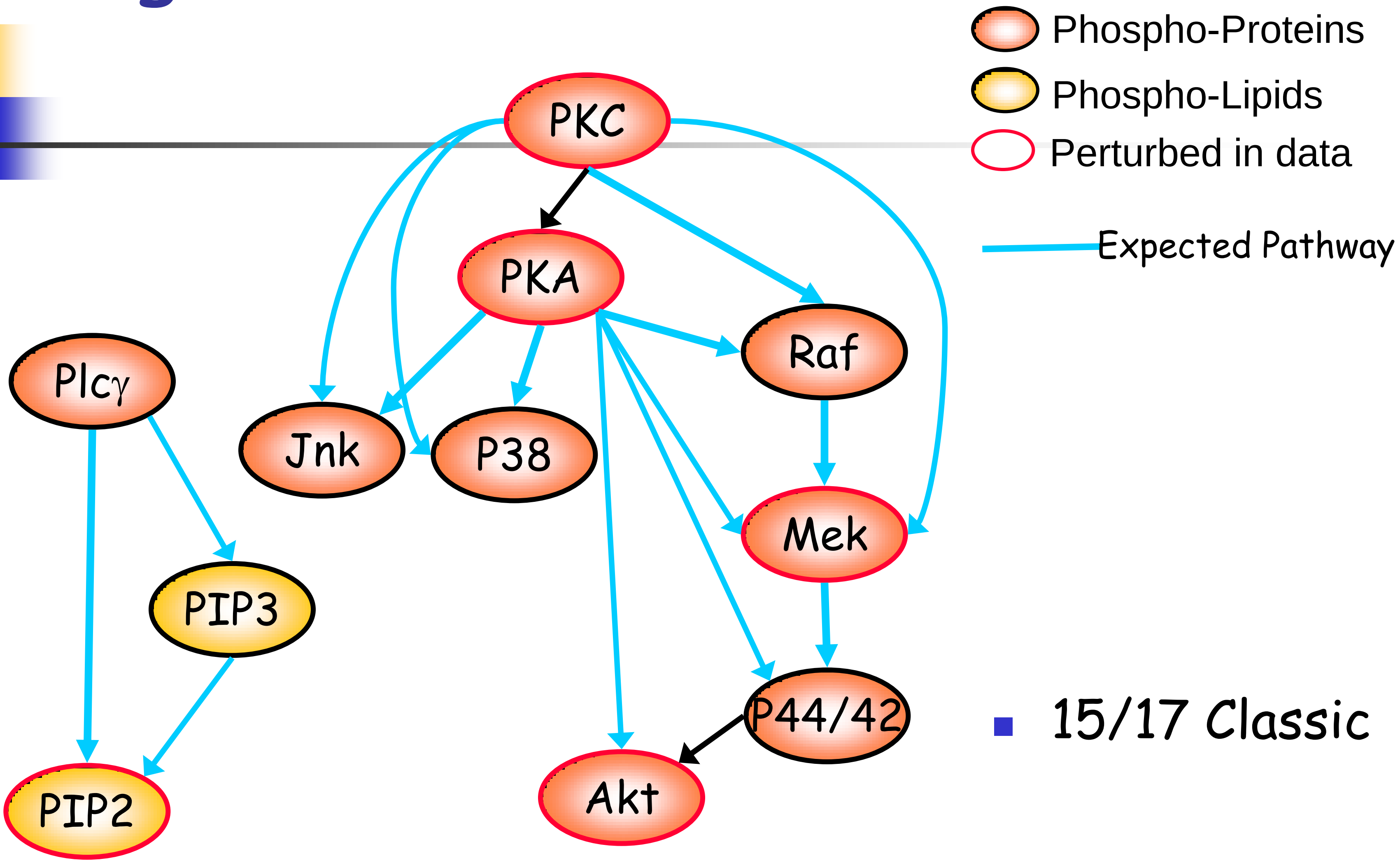


- The real picture



- Phospho-protein specific
- More than one pathway of influence

How good is the learned network?



- 15/17 Classic

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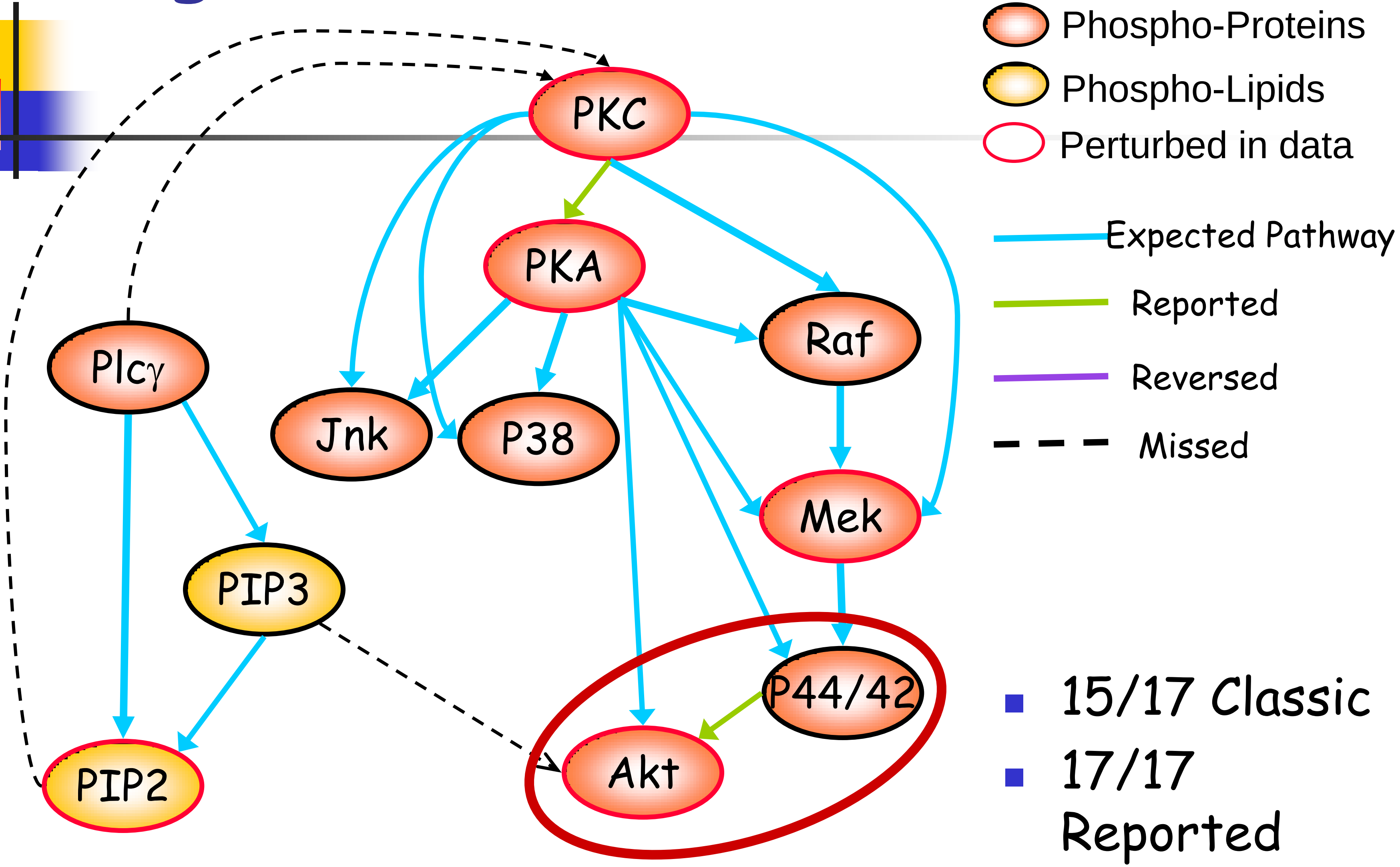
How good is the learned network?



- 15/17 Classic
- 17/17 Reported
- 3 Missed

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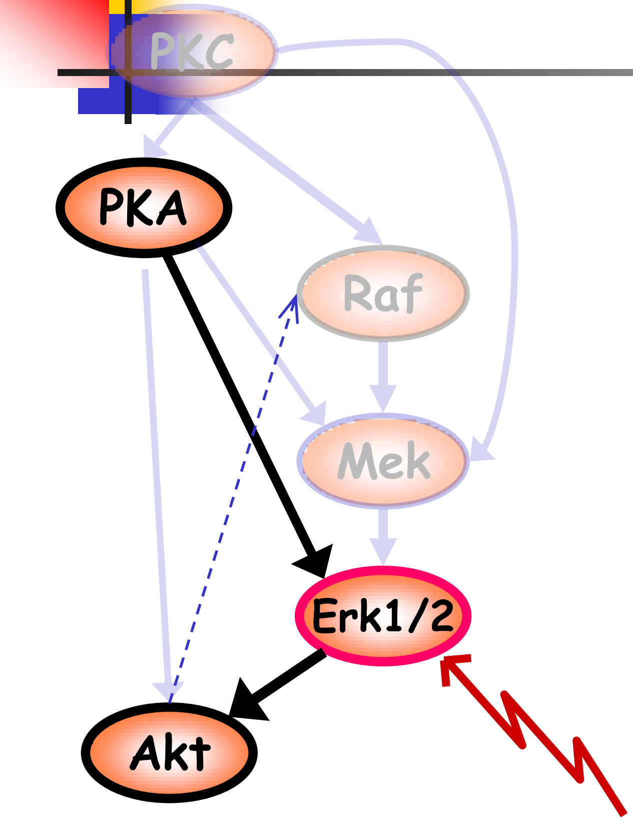
(c) Devika Subramanian, 2009

Prediction



- Erk influence on Akt previously reported in colon cancer cell lines

Predictions:

- Erk1/2 influences Akt
- While correlated, Erk1/2 does not influence PKA

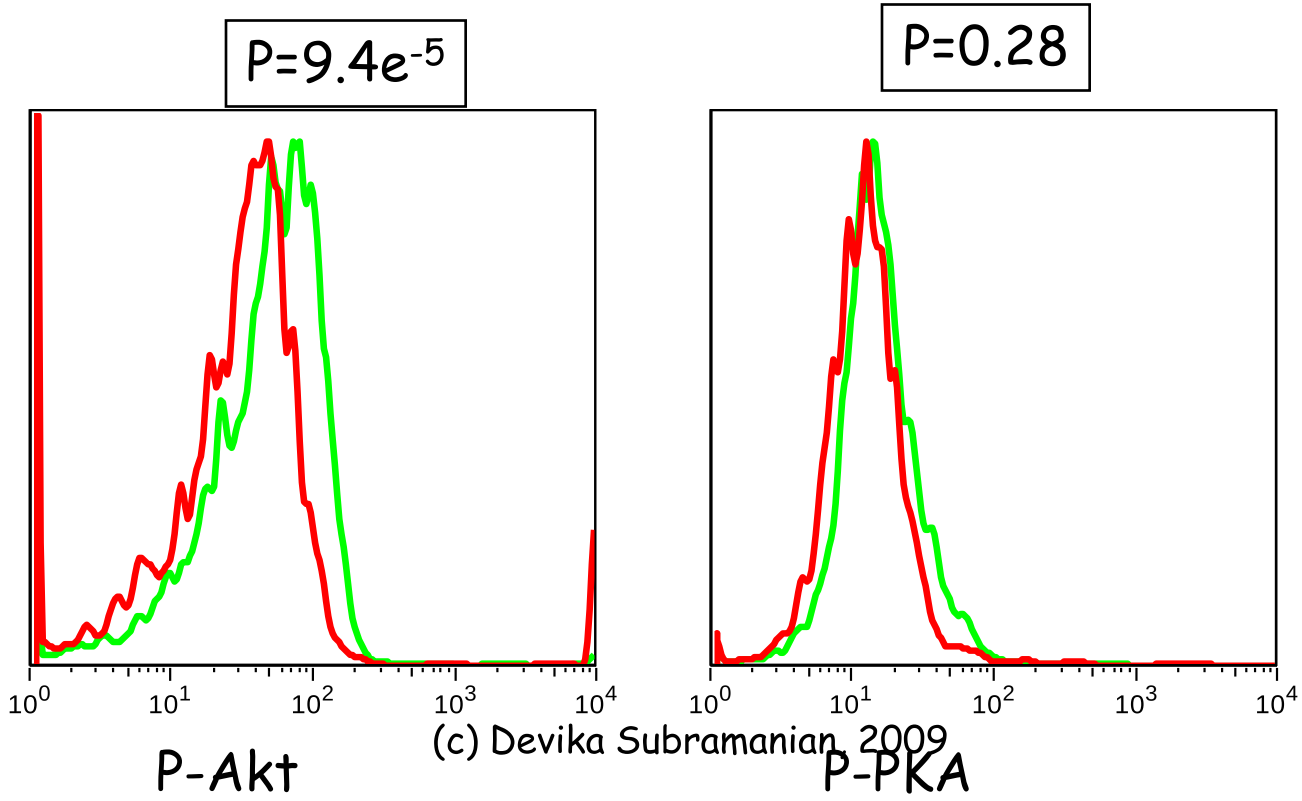
Sachs 2005

Validation

- SiRNA on Erk1/Erk2
- Select transfected cells
- Measure Akt and PKA

🟩 control, stimulated

🟥 Erk1 siRNA, stimulated



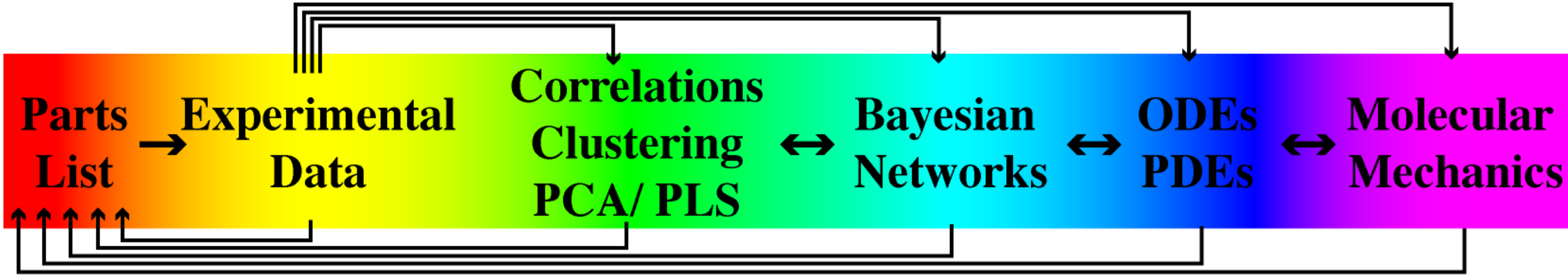
Sachs 2005

Summary

- Proof of principle: Automated reconstruction of signaling pathway in human cells
- Advantages:
  - In-vivo
  - Directed edges (causality)
  - Detects direct and in-direct influences
  - Single cell
  - Choose sub-populations of interest
- Disadvantage:
  - Static, cells fixed and stained
  - a-cyclic

Sachs et al, Science 2005

Spectrum of modeling tools in systems biology

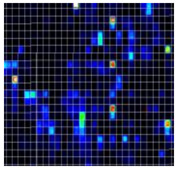


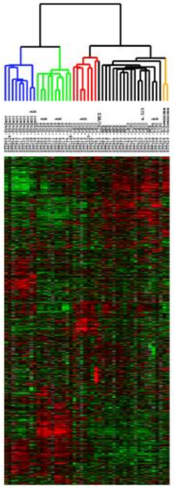
Search: LocusLink Display: Brief Organism: Human

Query: g-protein Go Clear

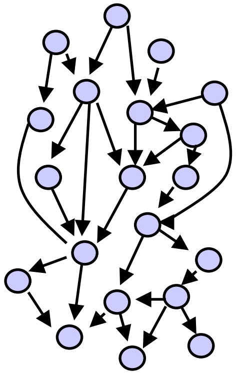
592 loci found This is page 1 of 12

LocusID	Org	Symbol	Description	Position	Links
□ 1636	Hs	ACE	angiotensin I converting enzyme (peptidyl-dipeptidase A) 1	17q23	P O R G P H U V
□ 117	Hs	ADCYAP1R1	adenylate cyclase activating polypeptide 1 (pituitary) receptor type I	7p14	P O R G P H U V
□ 11318	Hs	ADMR	adrenomedullin receptor	12q13.13	P O R G P H U V
□ 134	Hs	ADORA1	adenosine A1 receptor	1q32.1	P O R G P H U V
□ 135	Hs	ADORA2A	adenosine A2a receptor	22q11.23	P O R G P H U V
□ 136	Hs	ADORA2B	adenosine A2b receptor	17p12-p11.2	P O R G P H U V
□ 140	Hs	ADORA3	adenosine A3 receptor	1p21-p13	P O R G P H U V
□ 148	Hs	ADRA1A	adrenergic, alpha-1A-, receptor	8p21-p11.2	P O R G P H U V
□ 147	Hs	ADRA1B	adrenergic, alpha-1B-, receptor	5q23-q32	P O R G P H U V
□ 146	Hs	ADRA1D	adrenergic, alpha-1D-, receptor	20p13	P O R G P H U V
□ 150	Hs	ADRA2A	adrenergic, alpha-2A-, receptor	10q24-q26	P O R G P H U V
□ 151	Hs	ADRA2B	adrenergic, alpha-2B-, receptor	2p13-q13	P O R G P H U V
□ 152	Hs	ADRA2C	adrenergic, alpha-2C-, receptor	4p16	P O R G P





SVMs



$$d[R]/dt = k_1[LR] - k_2[R][L]$$

$$\ldots$$