

Systems biology

Comparative evaluation of reverse engineering gene regulatory networks with relevance networks, graphical gaussian models and bayesian networks

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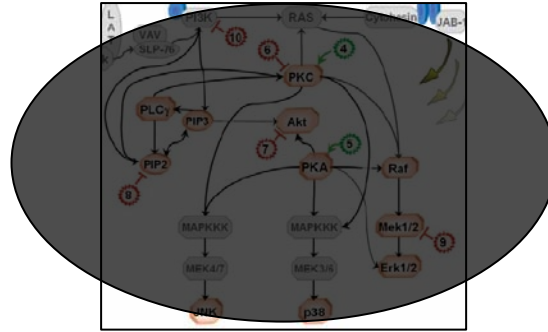
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Received on May 19, 2006; revised on July 7, 2006; accepted on July 10, 2006

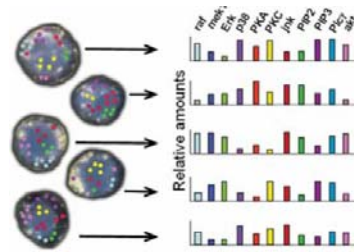
Advance Access publication July 14, 2006

Associate Editor: John Quackenbush

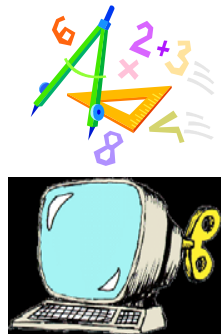
unknown



E.g.: Flow cytometry experiments

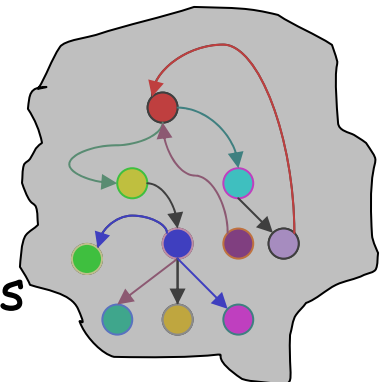


data data



machine learning

statistical methods



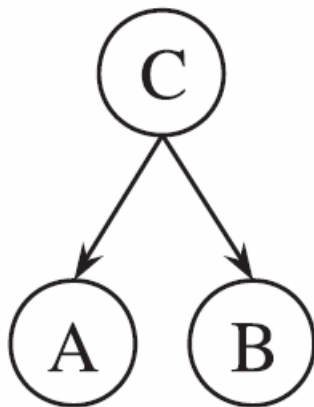
Reverse Engineering of Regulatory Networks

- Can we **learn** the **network** structure **from** postgenomic **data** themselves?
- Statistical methods to distinguish between
 - Direct interactions
 - Indirect interactions
- Challenge: Distinguish between
 - Correlations
 - Causal interactions
- Breaking symmetries with active interventions:
 - Gene knockouts (VIGs, RNAi)

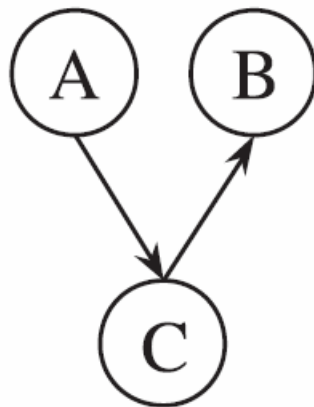
direct
interaction



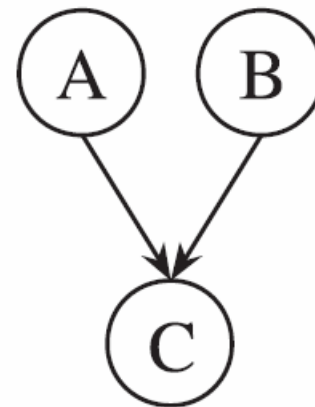
common
regulator



indirect
interaction

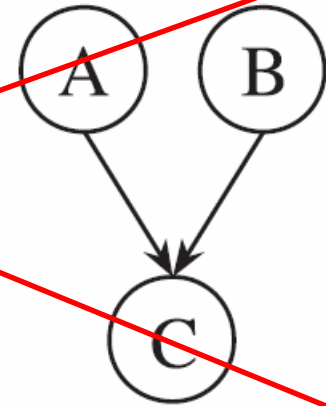
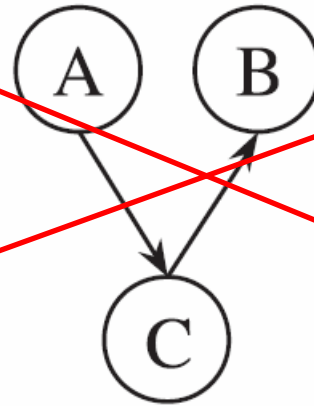
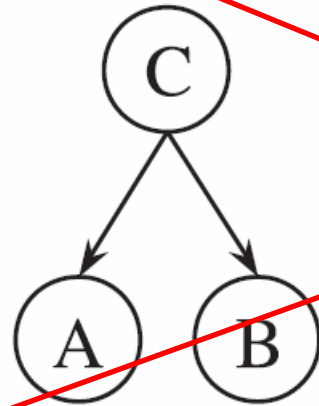


co-regulation



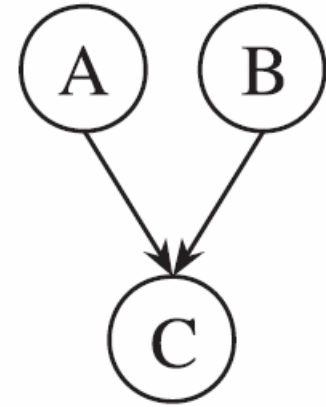
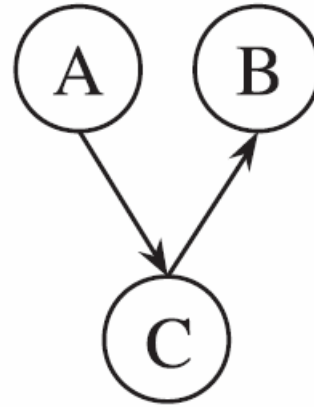
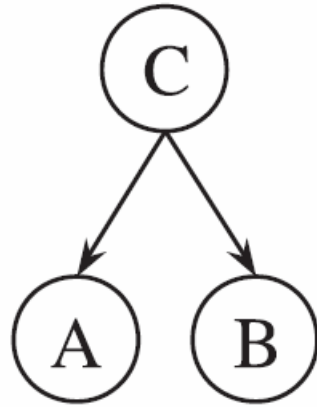
- Relevance networks
- Graphical Gaussian models
- Bayesian networks

Disadvantage of relevance networks



Cannot distinguish between **direct** and **indirect** interactions

Bayesian networks versus Graphical Gaussian models



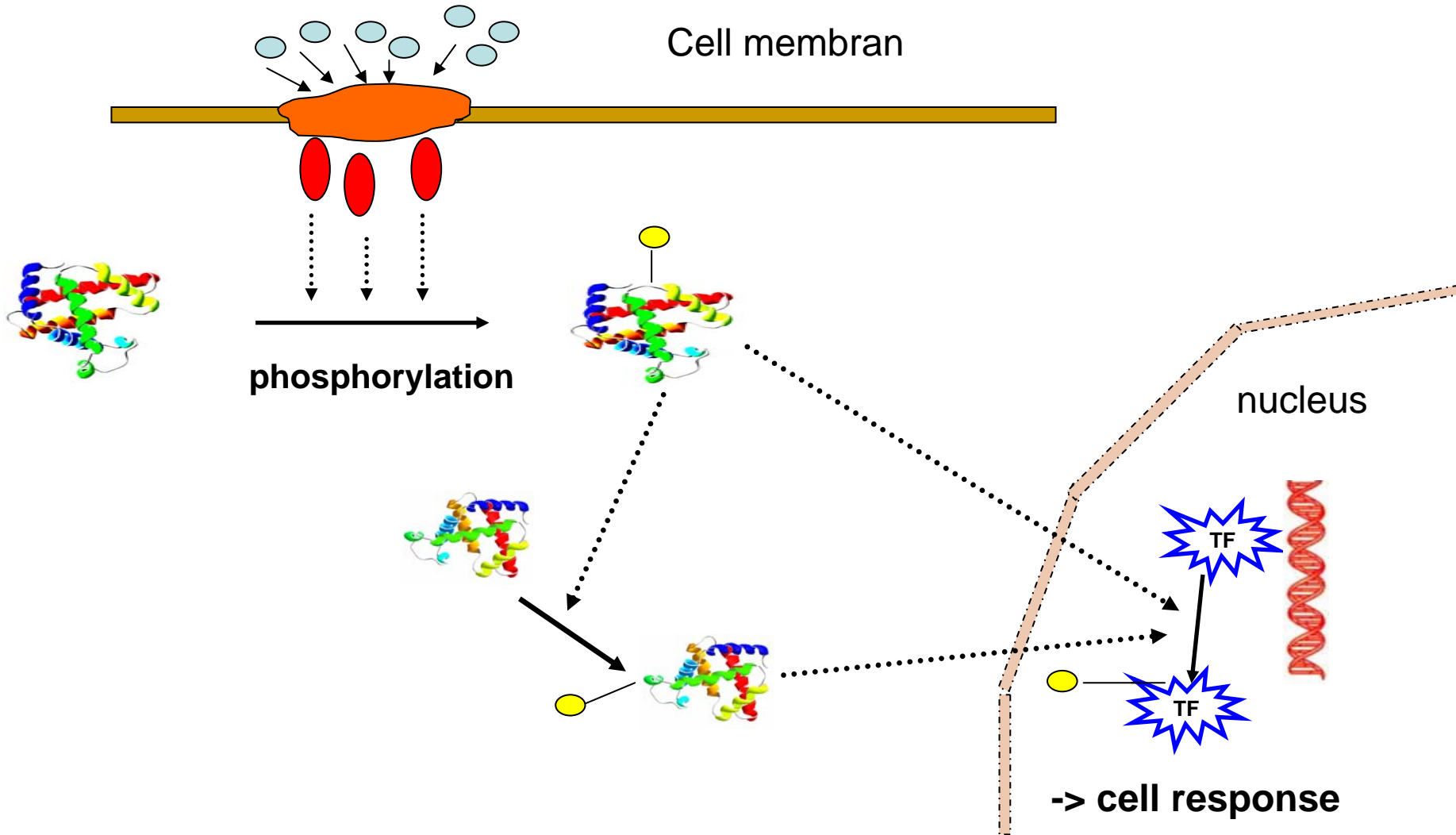
Directed versus undirected graphs

Score based versus constrained based inference

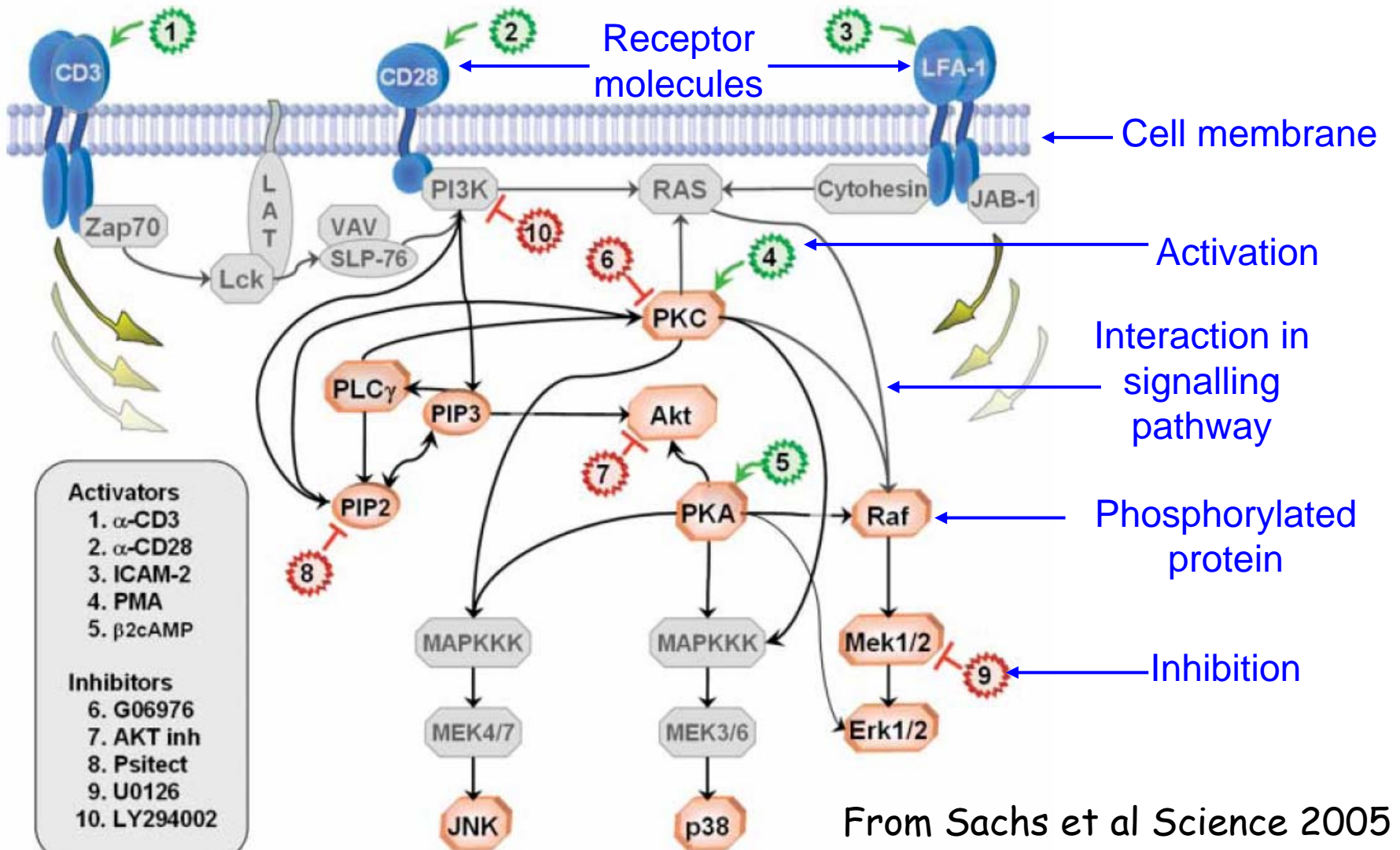
Biology

- Protein signal transduction pathway
 - Measuring protein concentrations with flow cytometry
 - Active interventions

Example: Protein signalling pathway

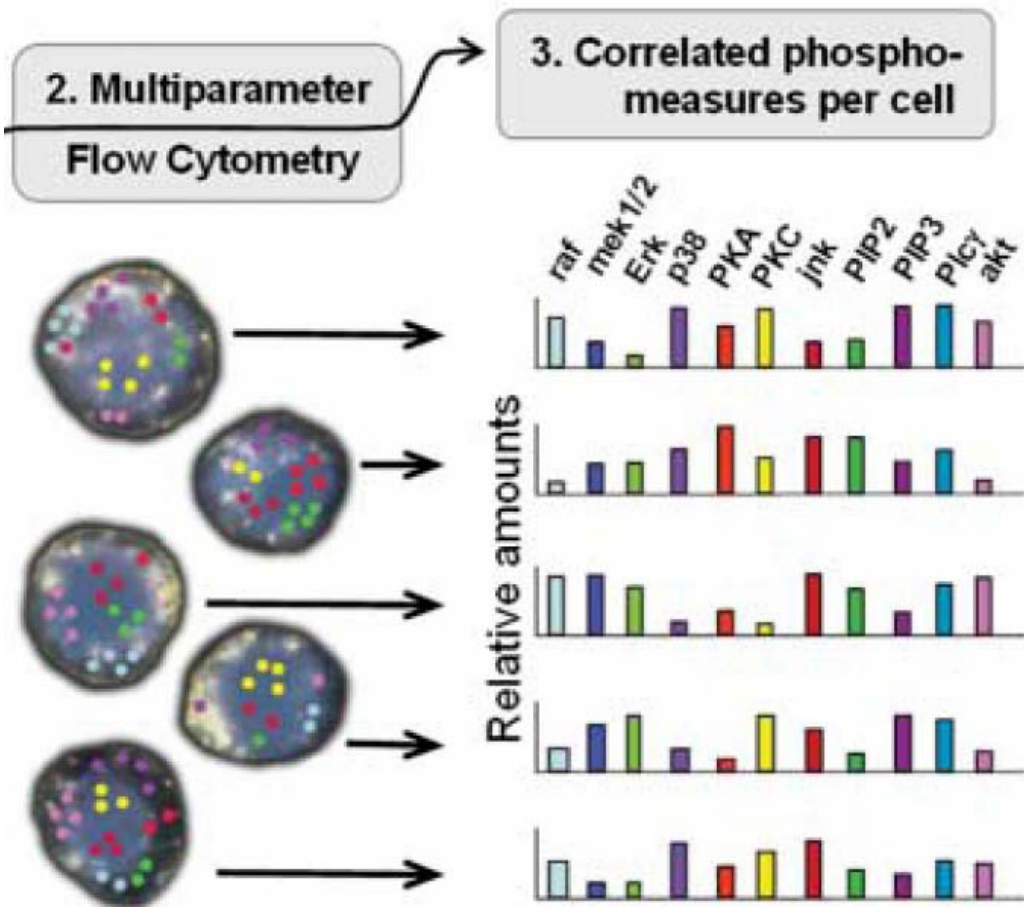


Raf signalling pathway



From Sachs et al Science 2005

Flow cytometry data



Causal Protein-Signaling Networks Derived from Multiparameter Single-Cell Data

Karen Sachs,^{1*} Omar Perez,^{2*} Dana Pe'er,^{3*}
Douglas A. Lauffenburger,^{1†} Garry P. Nolan^{2†}

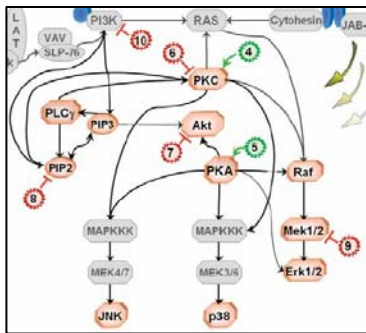
- Intracellular multicolour flow cytometry experiments: **concentrations of 11 proteins**
- **5400 cells** have been measured under 9 different cellular conditions (cues)
- **Downsampling** to 100 instances (5 separate subsets): **indicative of microarray experiments**

How do we assess the network reconstruction accuracy ?

- Real data → no gold standard
- Synthetic data → gold standard

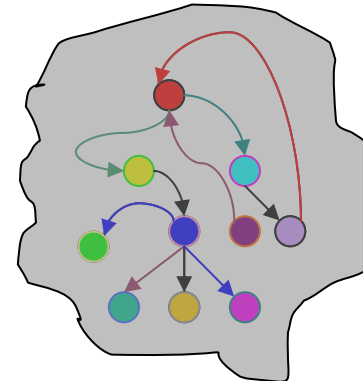
Assessing the network reconstruction accuracy

True network



Biological knowledge
(gold standard network)

Extracted network

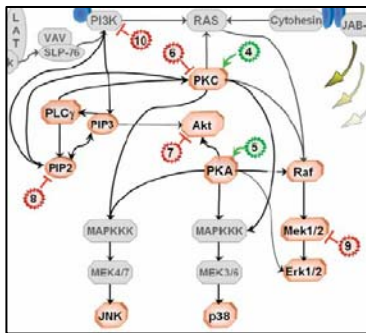


Evaluation of
performance



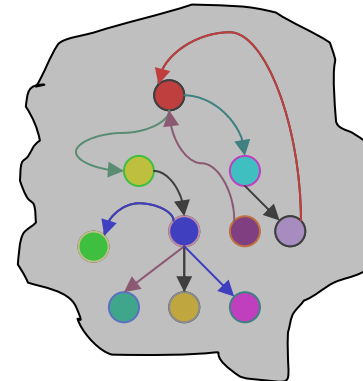
Assessing the network reconstruction accuracy

True network



↑
Biological knowledge
(gold standard network)

Extracted network



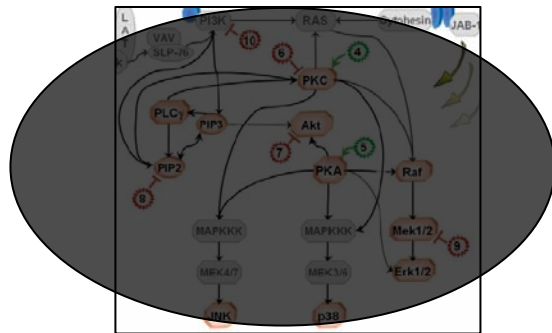
Evaluation of
performance



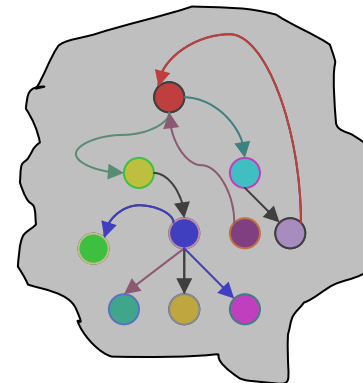
Feasible for
synthetic data

Assessing the network reconstruction accuracy

True network



Extracted network



Evaluation of performance



~~↑
Biological knowledge
(gold standard network)~~

Real data: take knowledge from the literature as a “bronze standard”

Evaluation

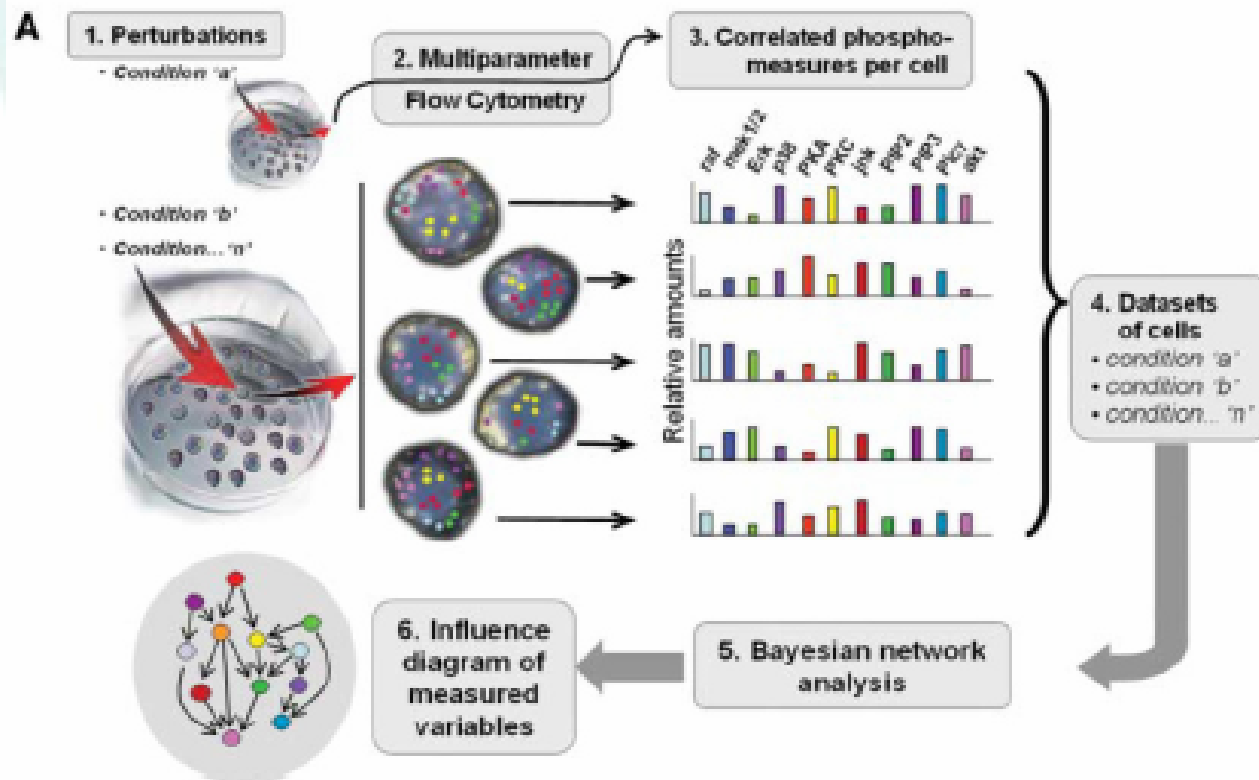
- On **real experimental** data, using the gold standard network from the literature
- On **synthetic data** simulated from the gold-standard network

Evaluation

- On real experimental data, using the gold standard network from the literature
- On synthetic data simulated from the gold-standard network

Causal Protein-Signaling Networks Derived from Multiparameter Single-Cell Data

Karen Sachs,^{1*} Omar Perez,^{2*} Dana Pe'er,^{3*}
Douglas A. Lauffenburger,^{1†} Garry P. Nolan^{2†}

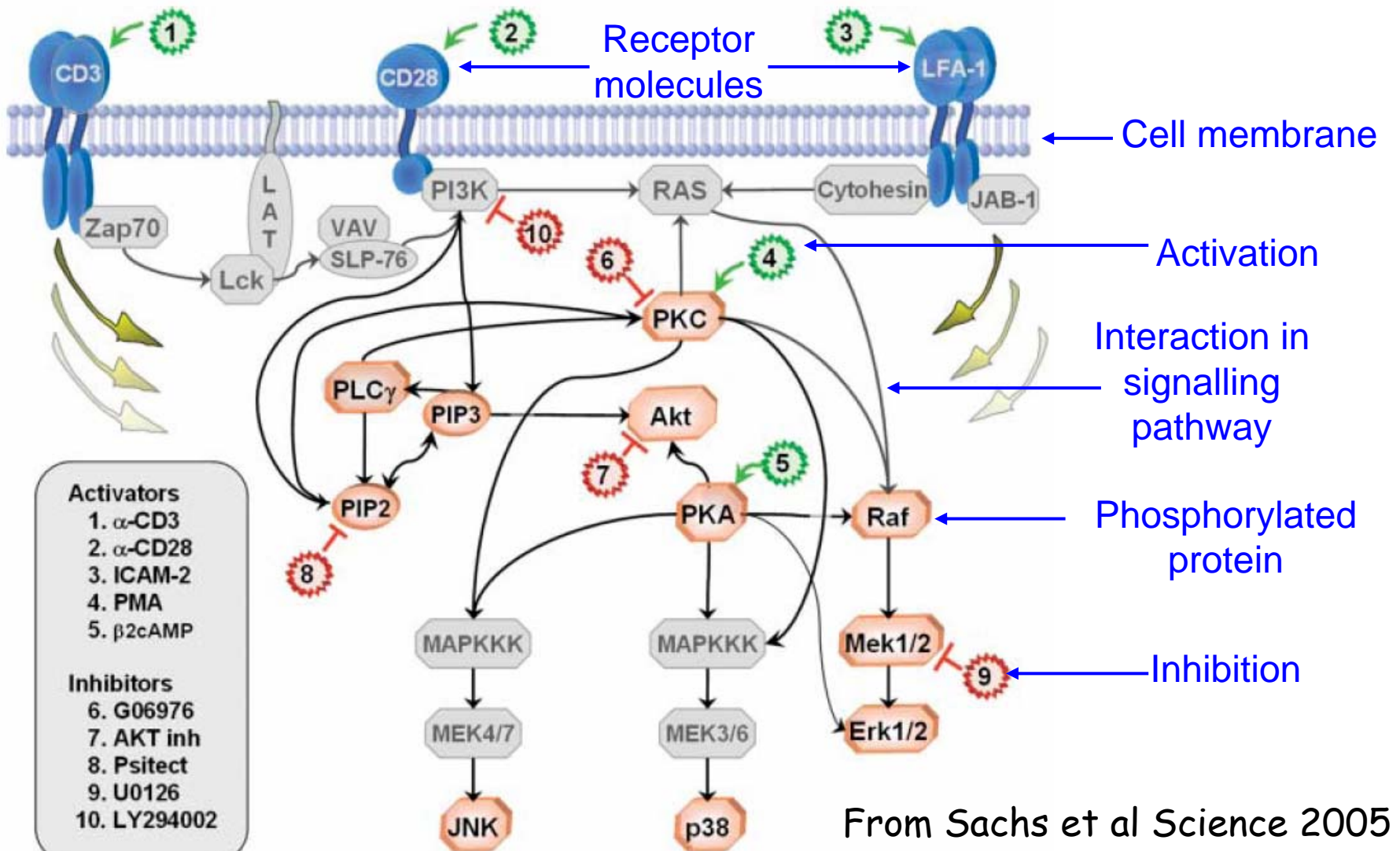


Evaluation:

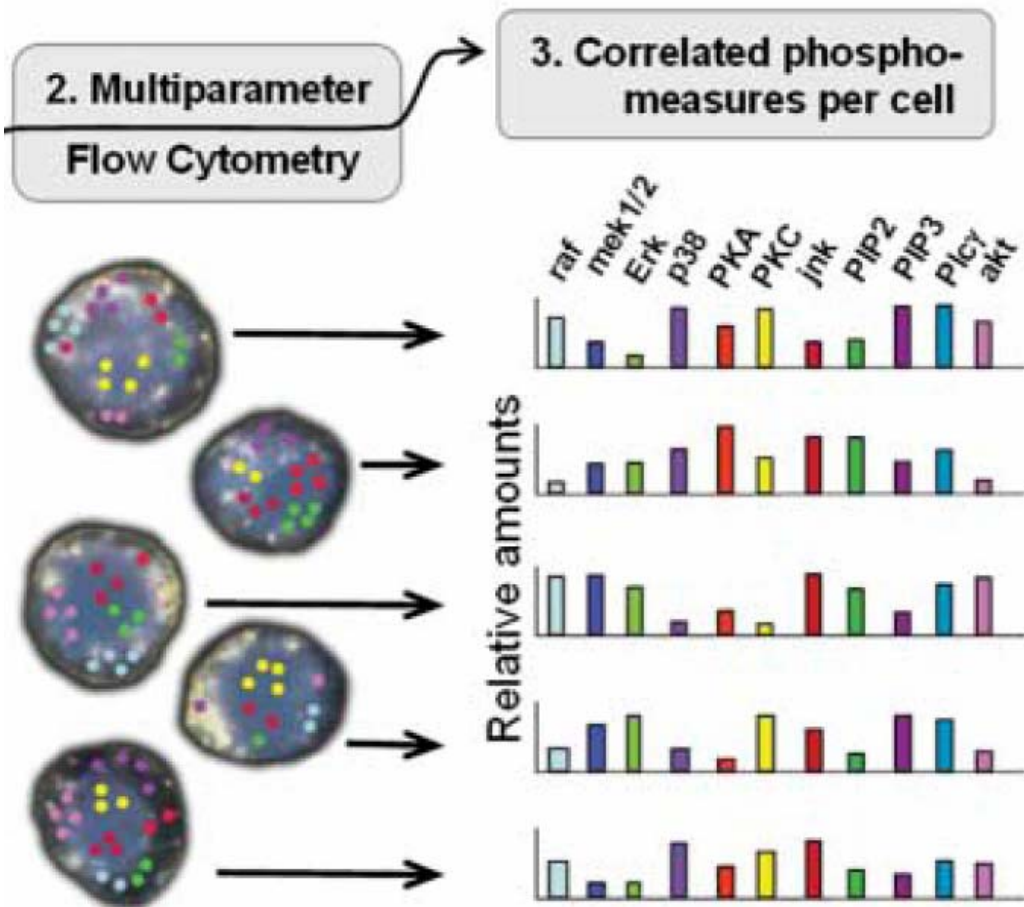
Raf signalling pathway

- Cellular signalling network of 11 phosphorylated proteins and phospholipids in human immune systems cell
- Deregulation → carcinogenesis
- Extensively studied in the literature → gold standard network

Raf signalling pathway



Data



Causal Protein-Signaling Networks Derived from Multiparameter Single-Cell Data

Karen Sachs,^{1*} Omar Perez,^{2*} Dana Pe'er,^{3*}
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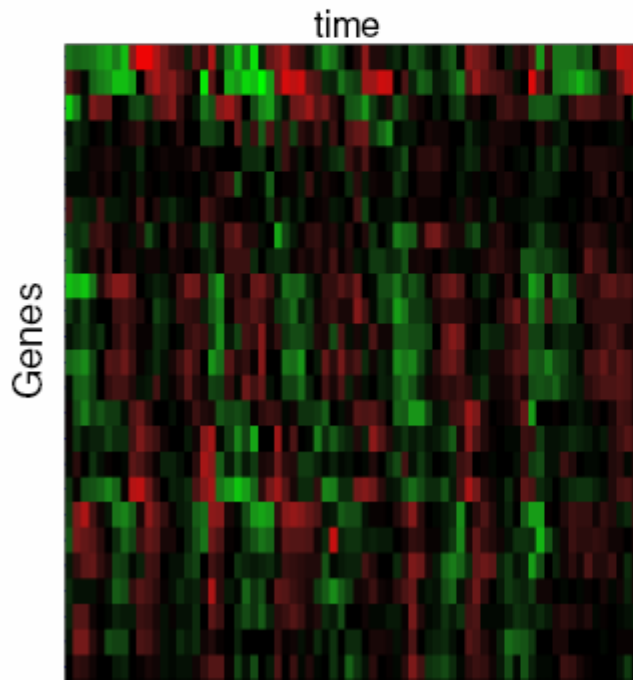
- Intracellular multicolour flow cytometry experiments concentrations of **11 proteins**
- **5400 cells** have been measured under 9 different cellular conditions (cues)
- We decided to **downsample** our test data sets to 100 instances - **indicative of microarray experiments**

Expression data: Microarray time series experiments

Spellman et al (1998)

Cell cycle

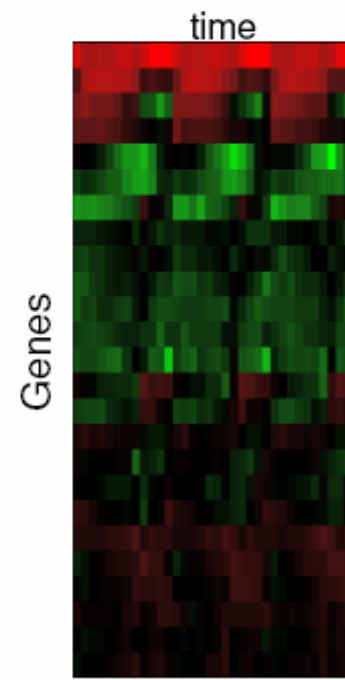
73 time points



Tu et al (2005)

Metabolic cycle

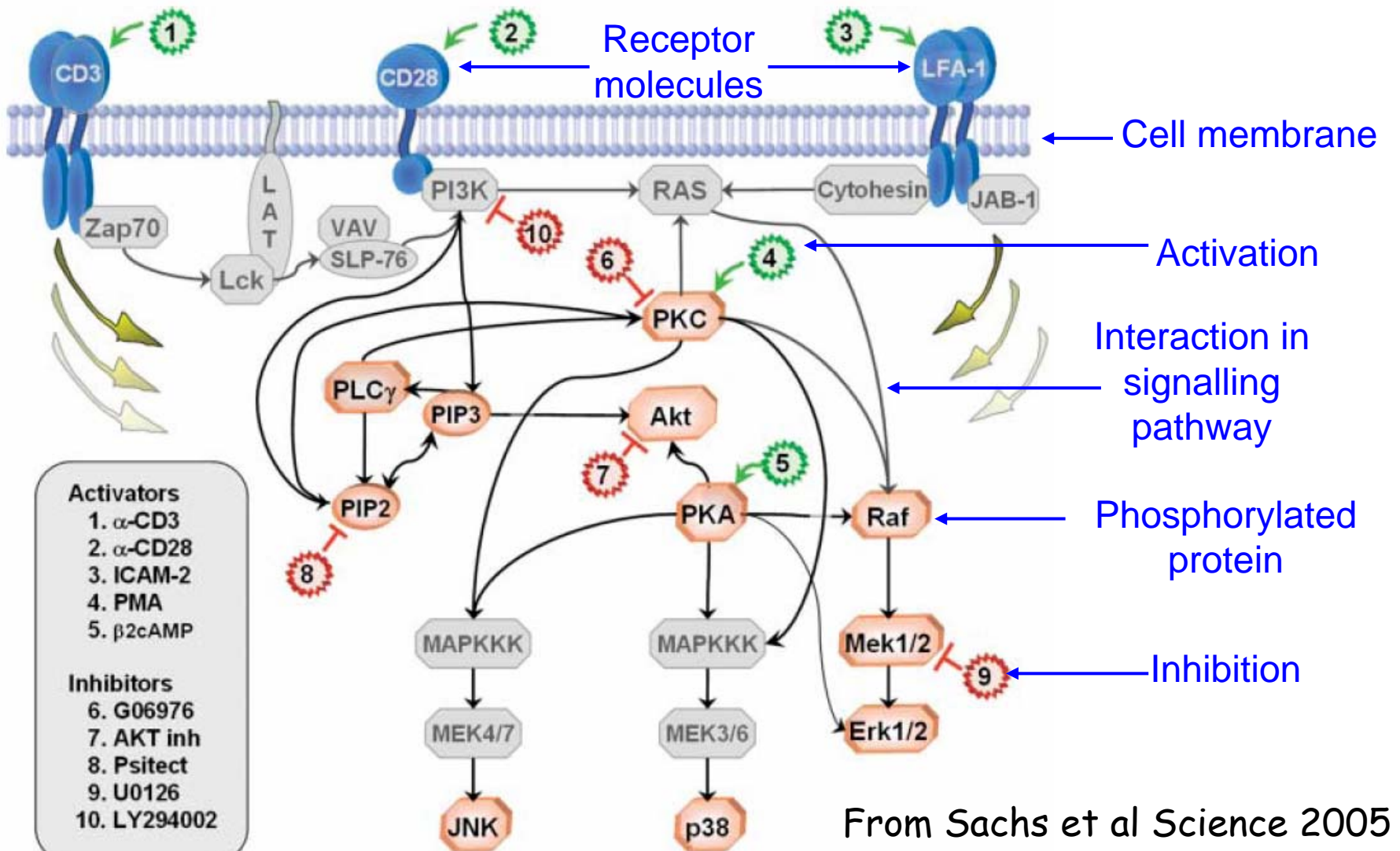
36 time points.



Two types of experiments

- **Observational data** are measurements obtained by passively monitoring the biological system without any interference.
- **Interventional data** are obtained by actively manipulating certain domain variables, e.g. using gene knock-outs or overexpressions.

Raf signalling pathway



From Sachs et al Science 2005

Evaluation

- On real experimental data, using the gold standard network from the literature
- On synthetic data simulated from the gold-standard network

Comparison with simulated data

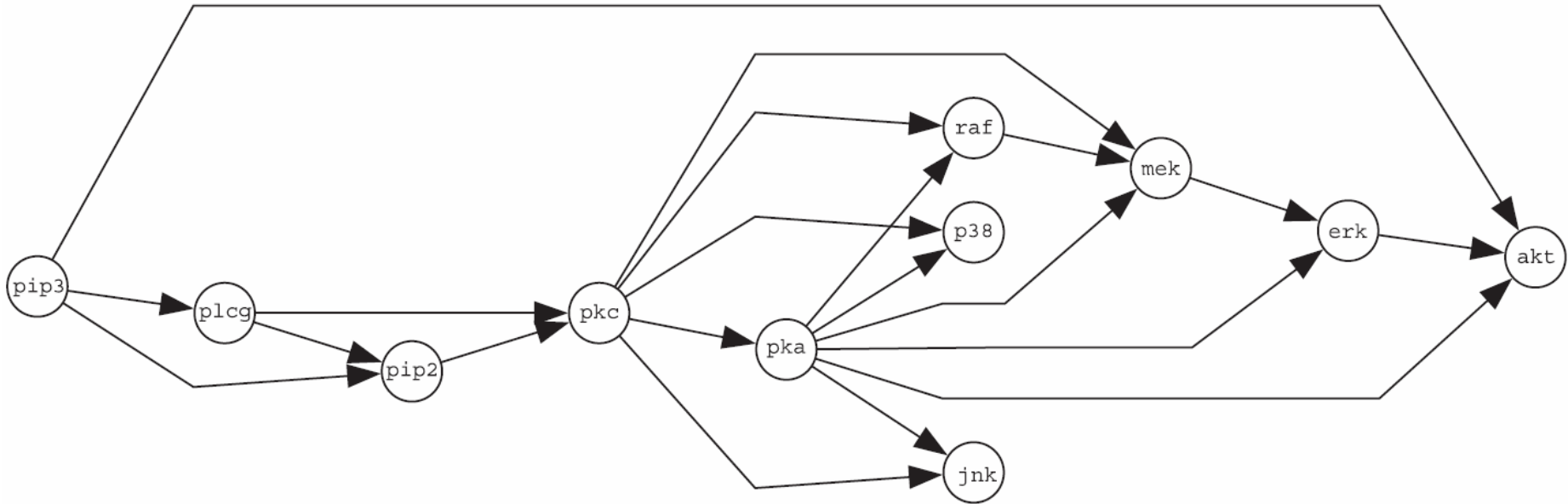
$$X_i \sim N\left(\sum_k w_{ik} x_k, \sigma\right)$$

$$\sigma = 0.1$$

$|w_{ik}|$ uniform distribution over the interval [0.5,2]

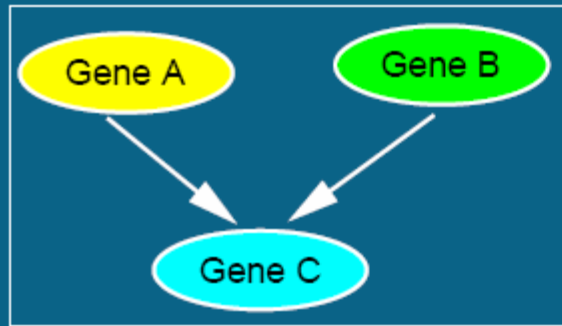
For inhibitions, we sampled X_i from a zero-mean Gaussian distribution, $N(0, \sigma)$. For activations, we sampled X_i from the tails of the empirical distribution of X_i , beyond the 2.5 and the 97.5 percentiles.

Raf pathway

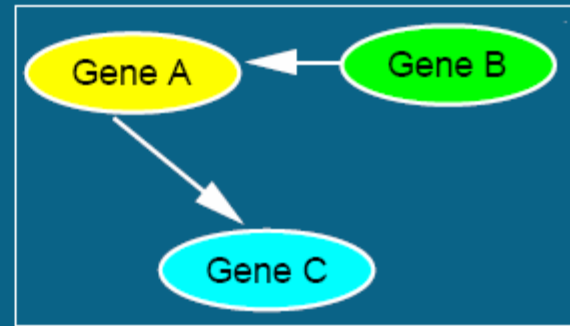


How do we measure the performance ?

- AUROC versus TPFP5
 - DGE versus UGE



compare

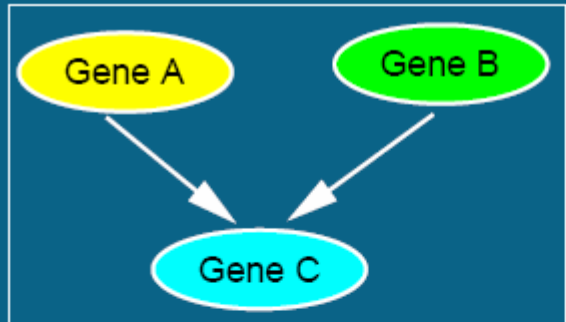


generate

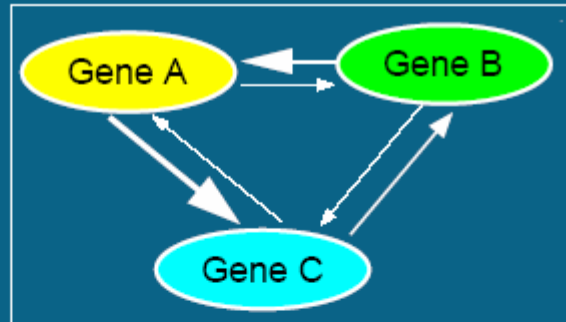
Data

learn

Deterministic inference



compare

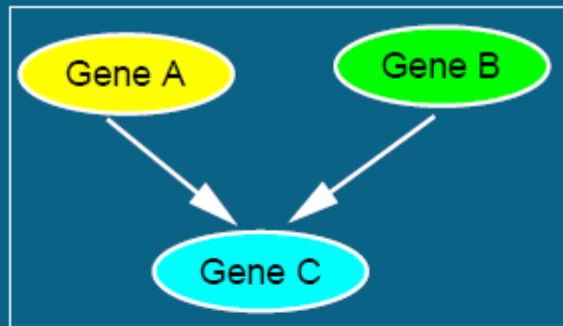


generate

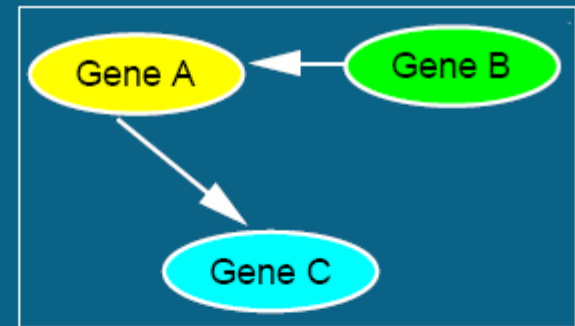
Data

learn

Probabilistic inference



compare



generate

Data

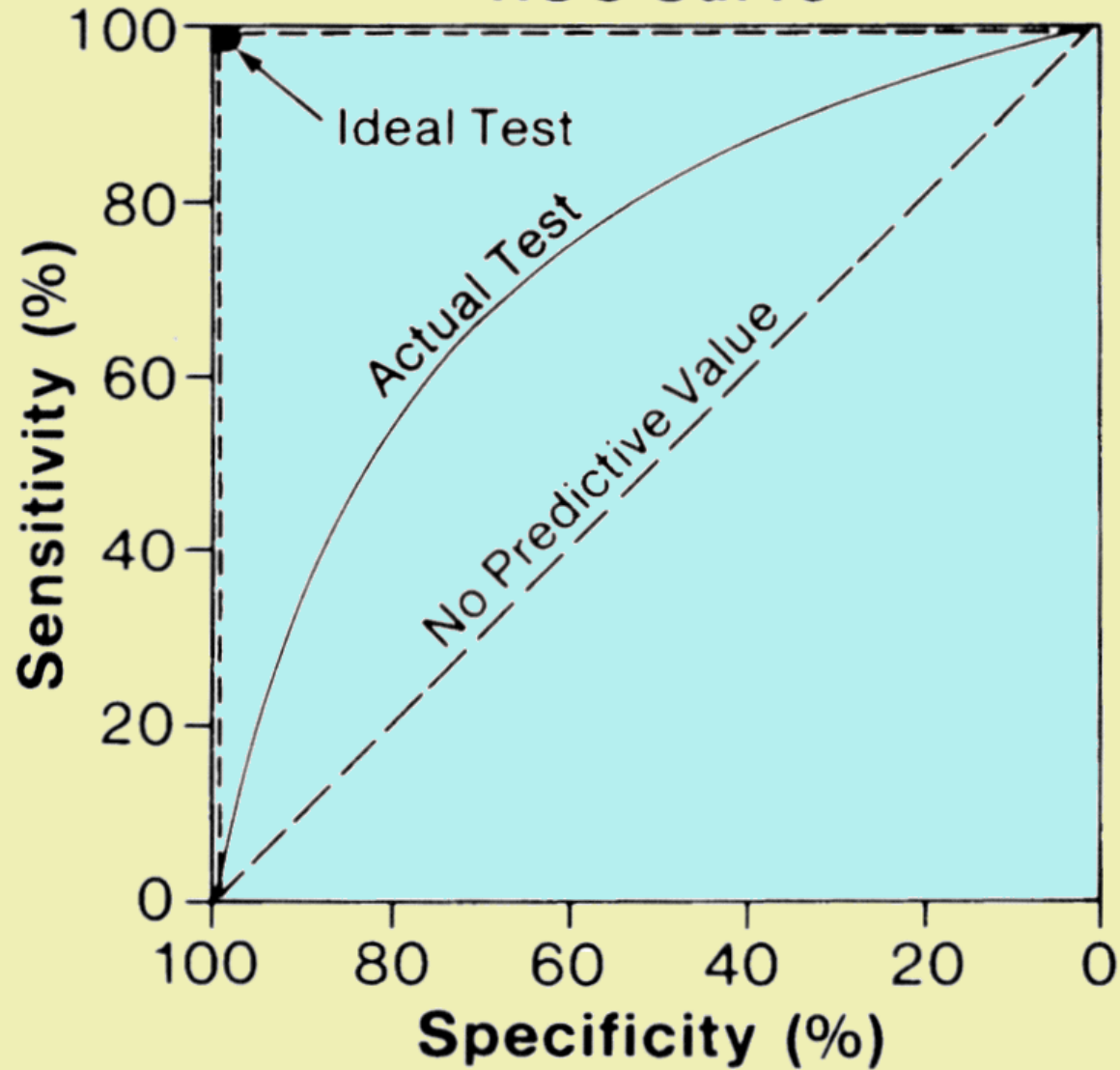
learn

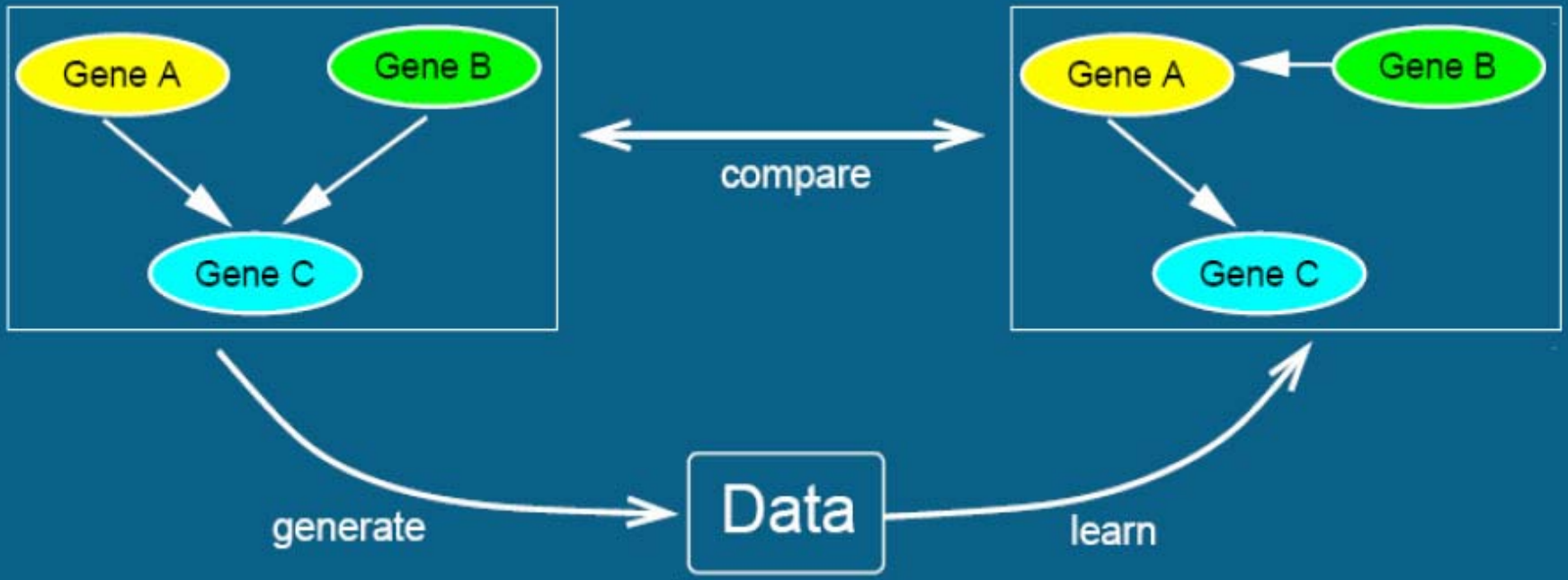
Thresholding

True positives

False positives

ROC Curve





True positives (TP) = ?

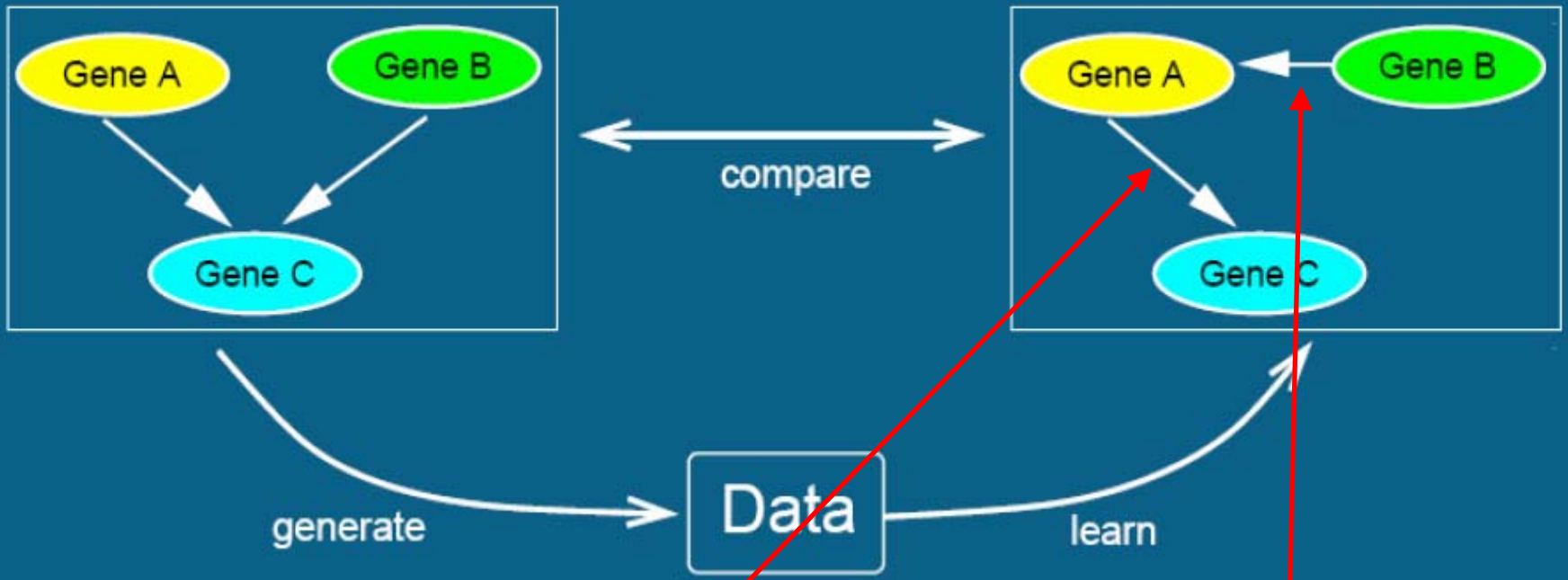
False positives (FP) = ?

False negatives (FN) = ?

True negatives (TN) = ?

Sensitivity = $TP / (TP + FN) = ?$

Specificity = $TN / (TN + FP) = ?$



True positives (TP) = 1

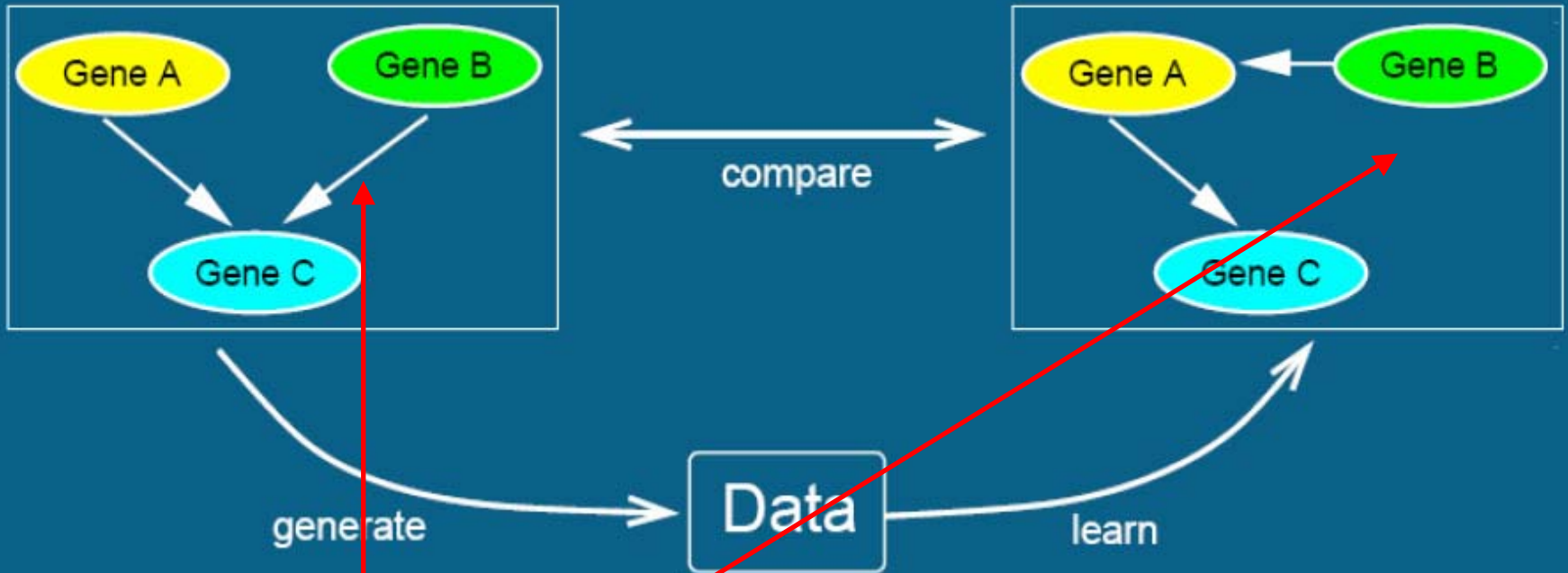
False negatives (FN) = ?

Sensitivity = $TP / (TP + FN) = ?$

False positives (FP) = 1

True negatives (TN) = ?

Specificity = $TN / (TN + FP) = ?$



True positives (TP) = 1

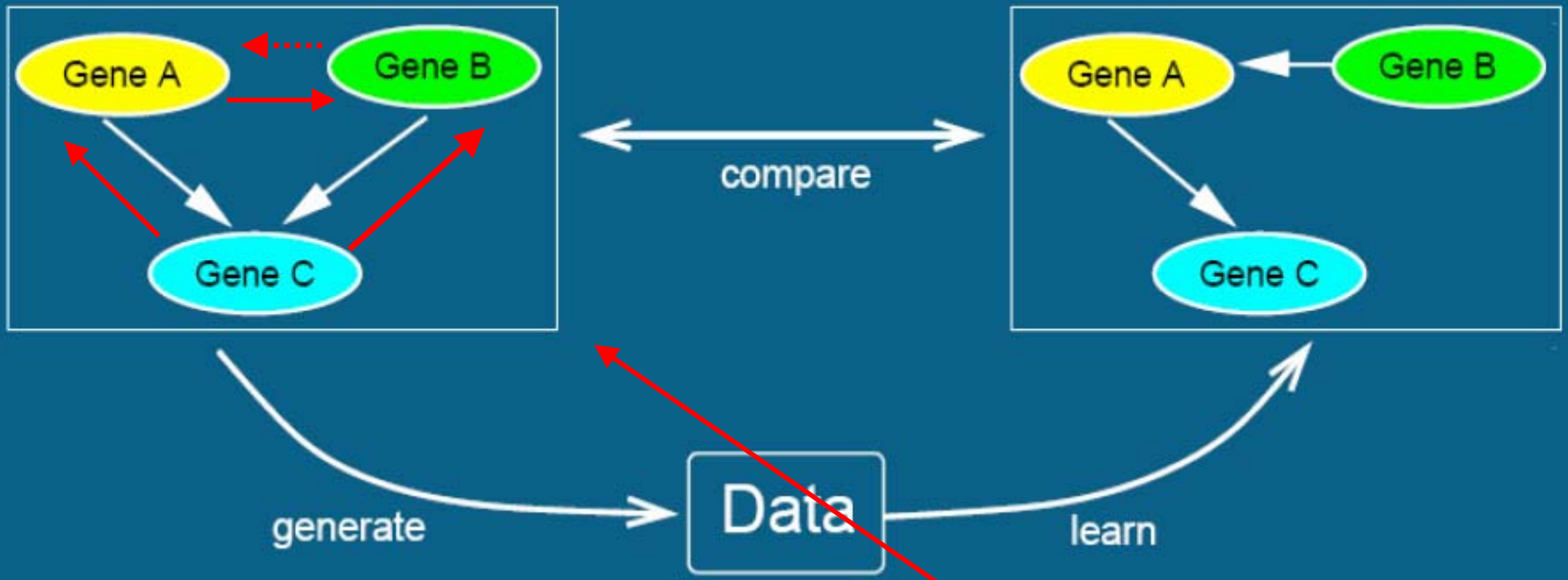
False negatives (FN) = 1

Sensitivity = $TP / (TP + FN) = ?$

False positives (FP) = 1

True negatives (TN) = ?

Specificity = $TN / (TN + FP) = ?$



True positives (TP) = 1

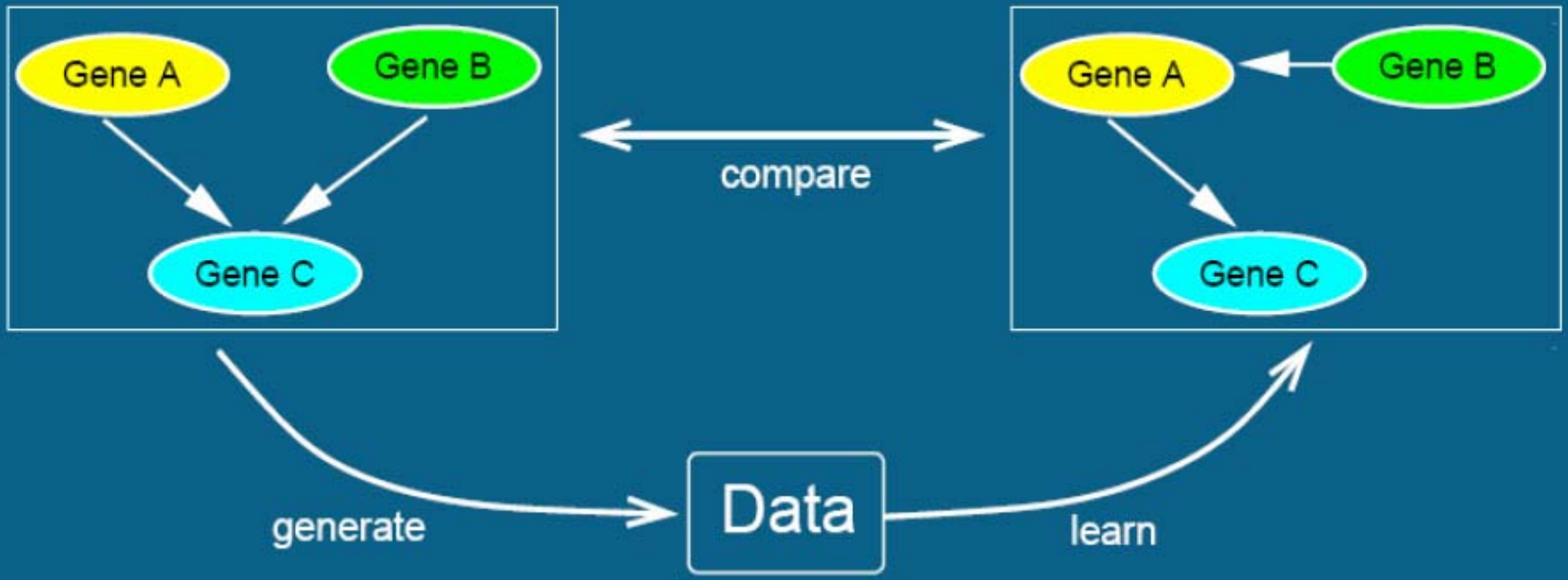
False negatives (FN) = 1

Sensitivity = $TP / (TP + FN) = ?$

False positives (FP) = 1

True negatives (TN) = 3

Specificity = $TN / (TN + FP) = ?$



True positives (TP) = 1

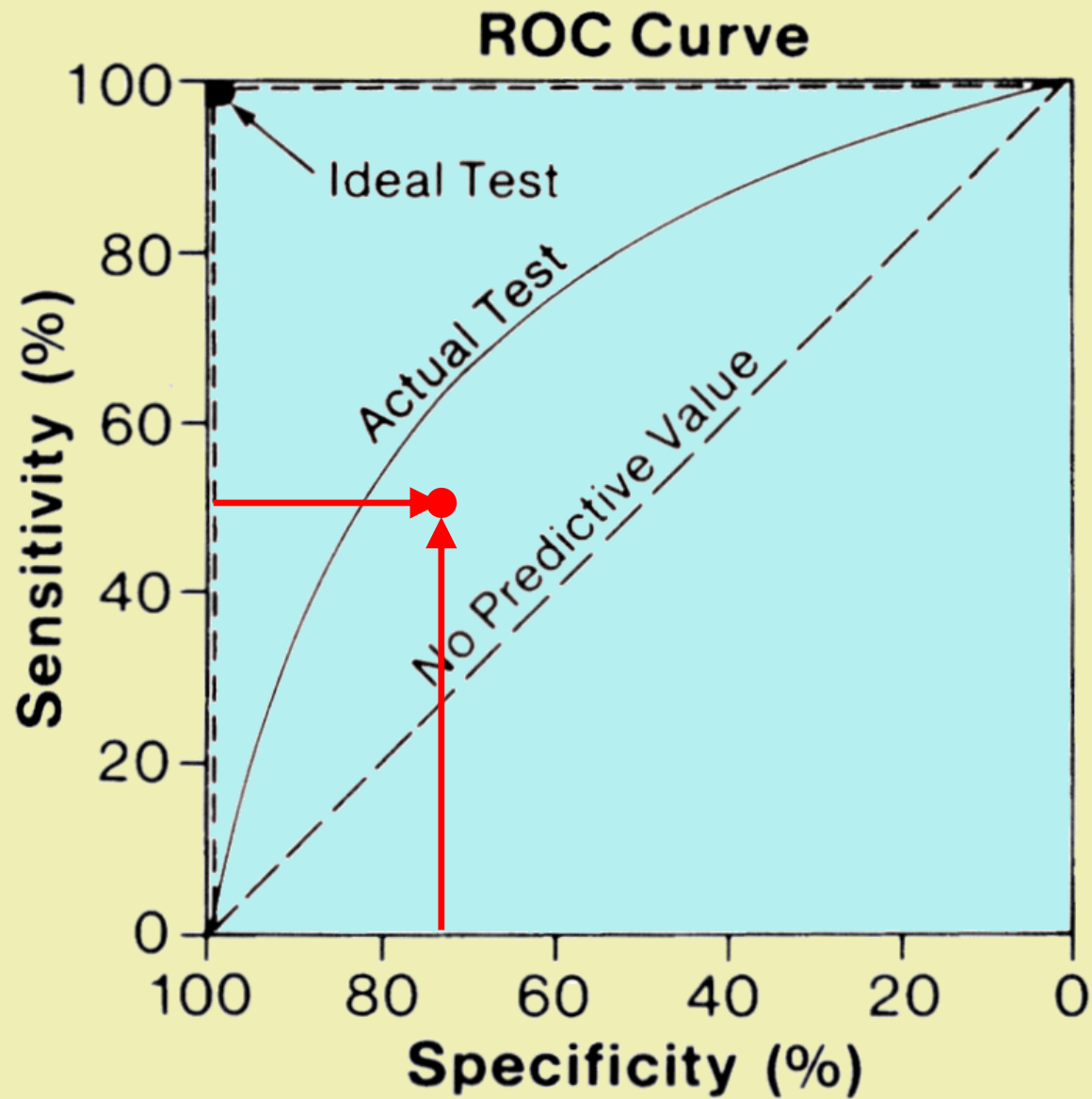
False positives (FP) = 1

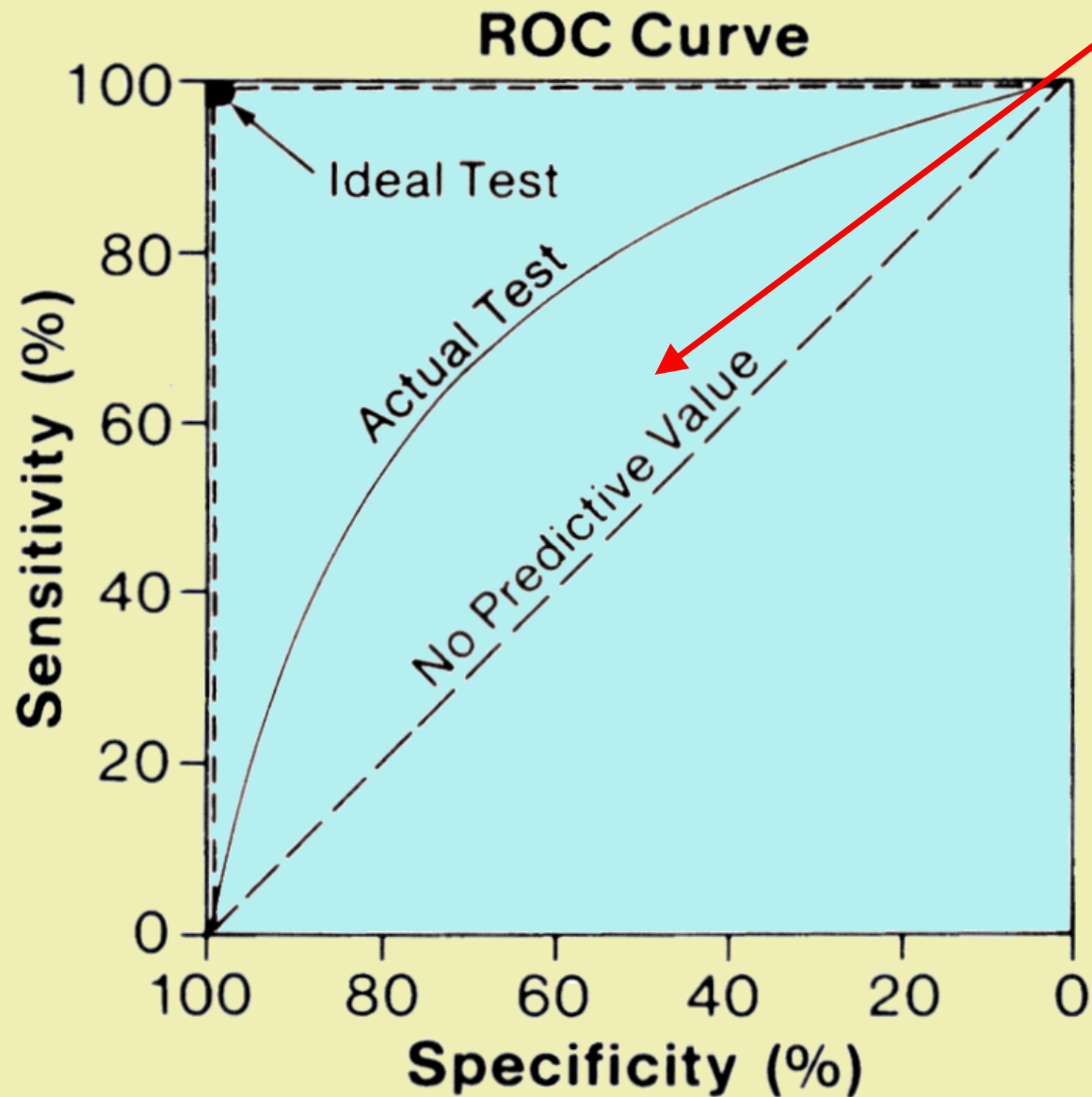
False negatives (FN) = 1

True negatives (TN) = 3

Sensitivity = $TP / (TP + FN) = 0.5$

Specificity = $TN / (TN + FP) = 0.75$





AUC:
area under
the curve.

AUC = 1:
perfect
prediction

AUC = 0.5:
random
expectation

Directed graph evaluation (DGE)

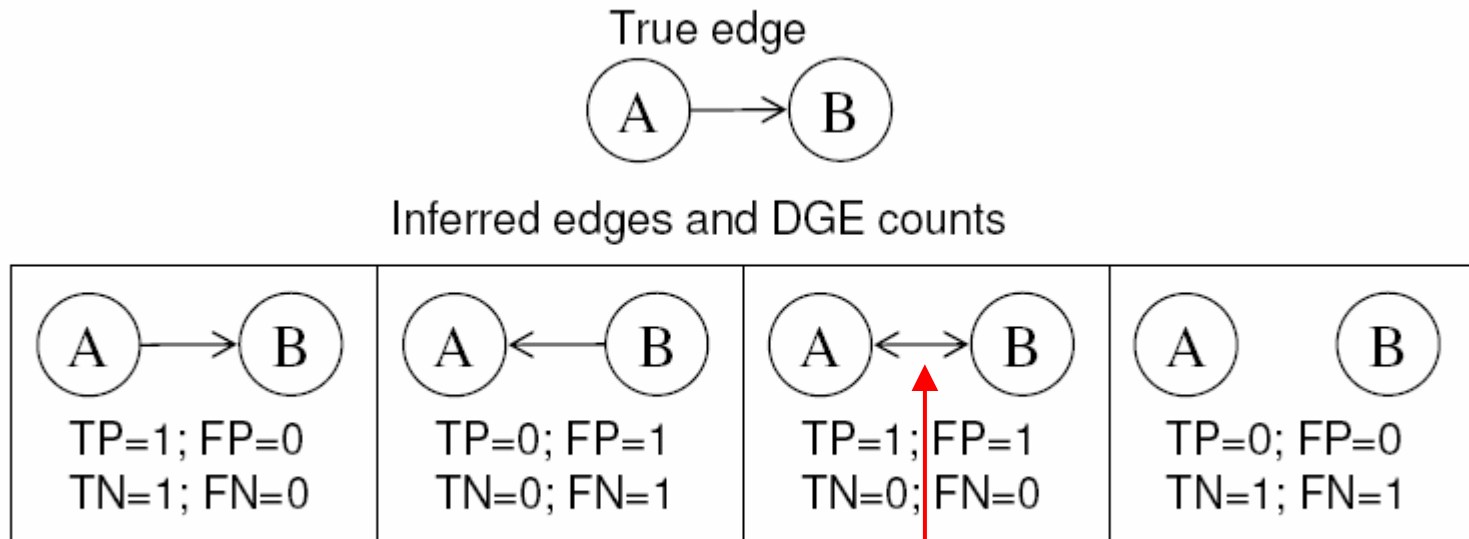
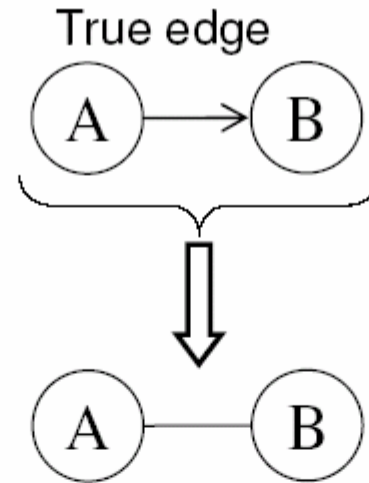


Figure 4.5: DGE Scoring. This schematic example shows how the *directed graph evaluation*

Undirected edge = superposition of
two directed edges

Undirected graph evaluation (UGE)



Inferred edges and UGE counts

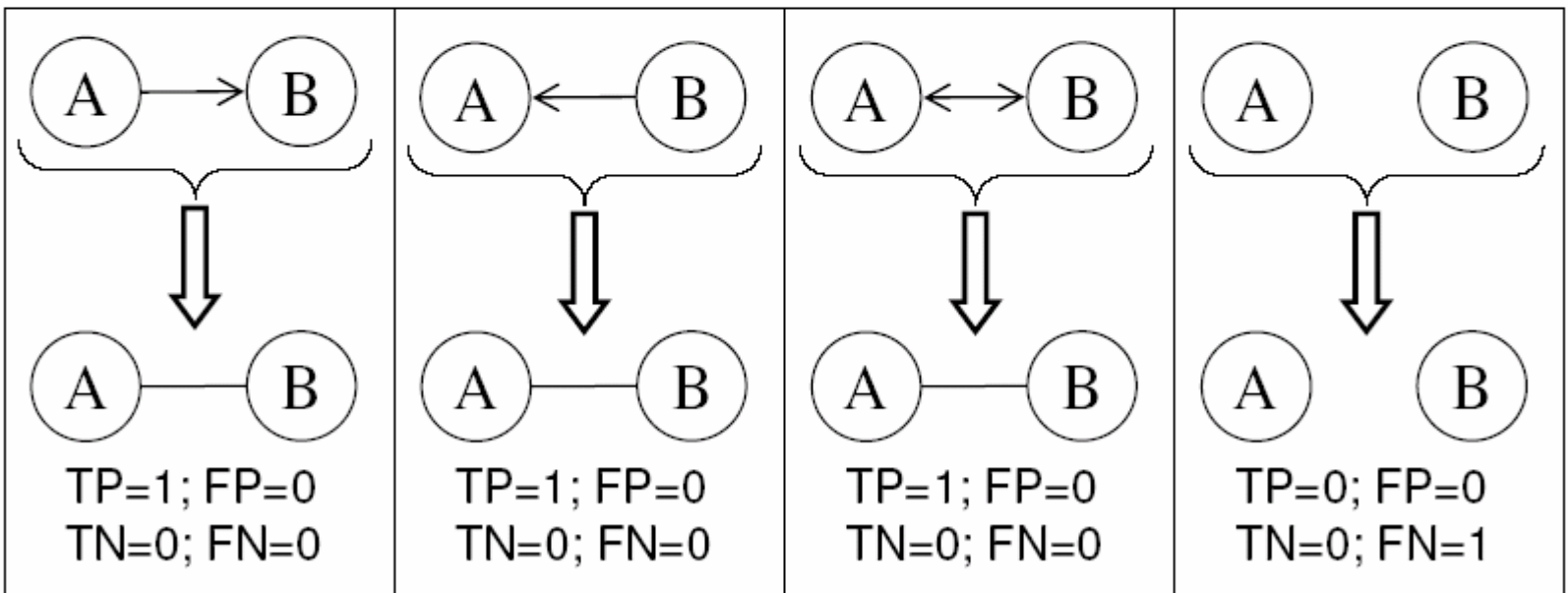
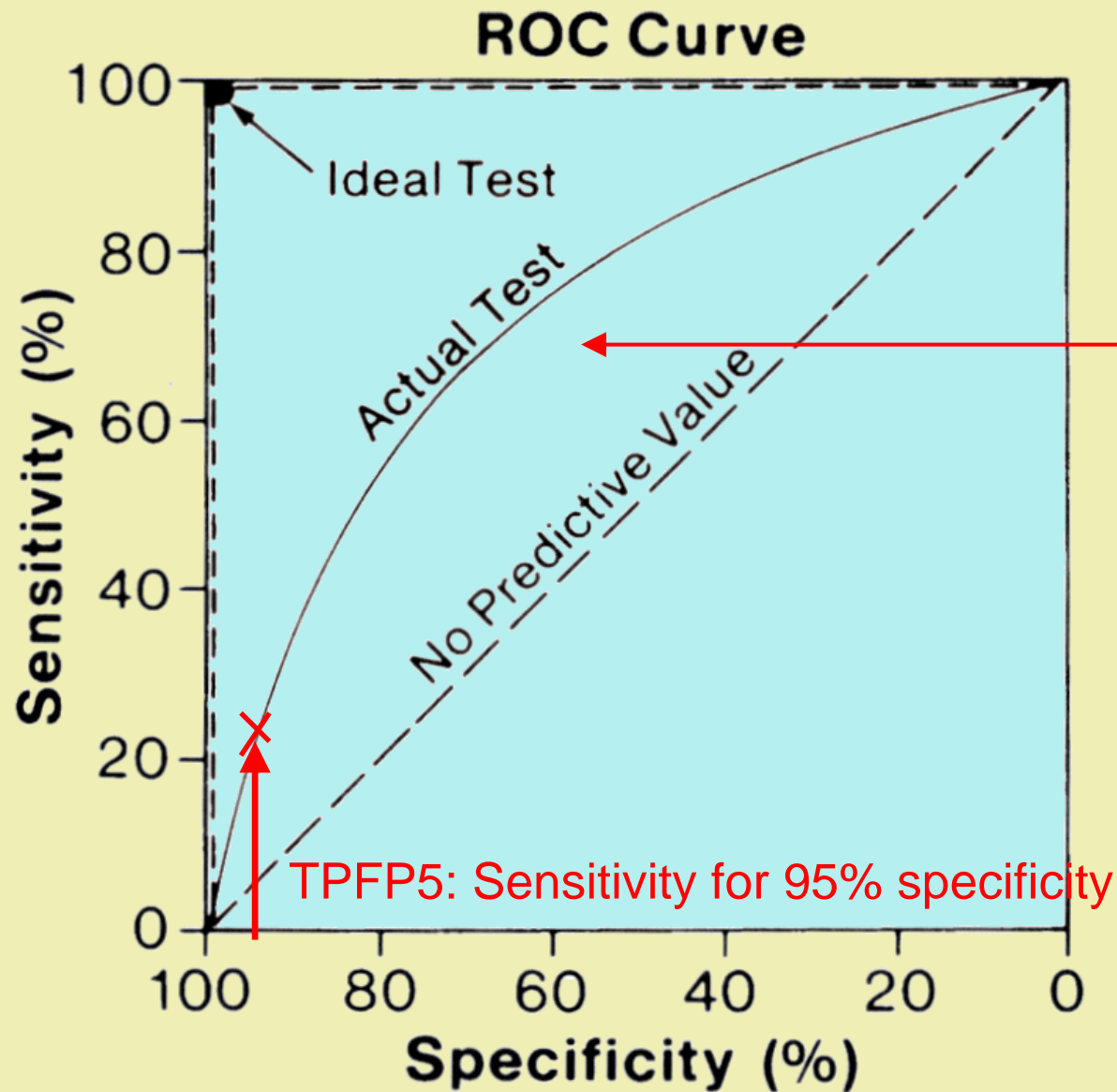


Figure 4.4: UGE scoring. This schematic example shows how the *undirected graph evaluation*



AUC:
area
under
the
curve

TPFP5: Sensitivity for 95% specificity

Evaluation

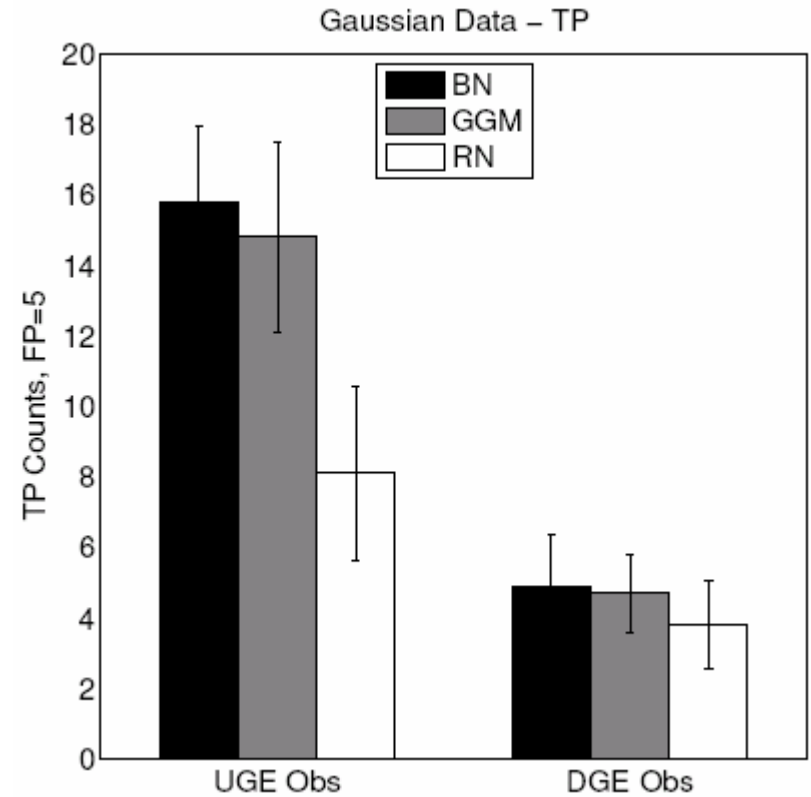
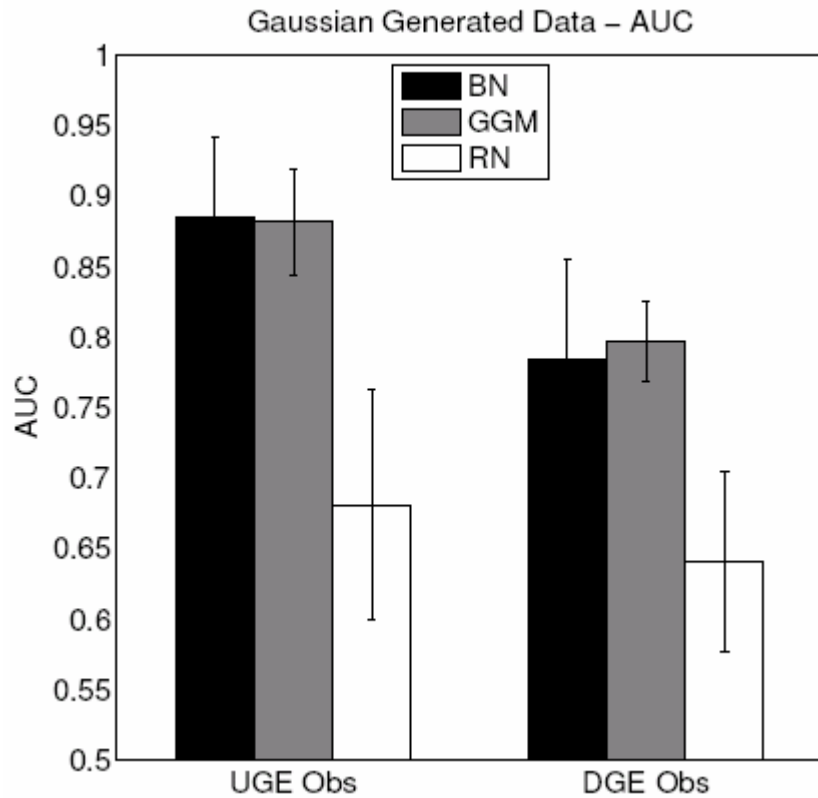
Two ways of interpreting edges

- **UGE:** undirected graph evaluation
- **DGE:** directed graph evaluation

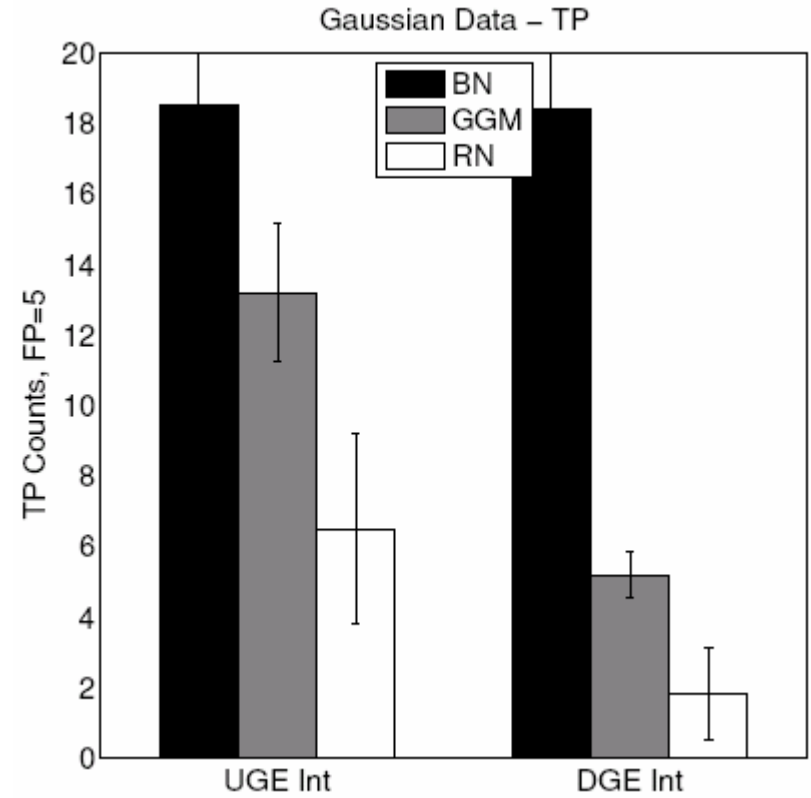
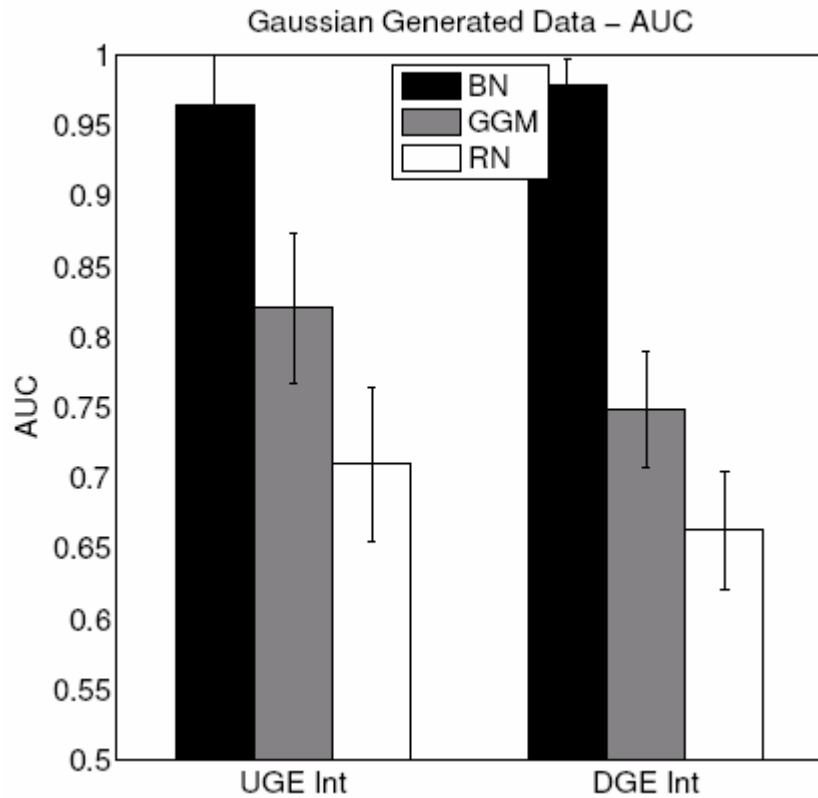
Two evaluation procedures:

- **AUC:** Area under the ROC curve, with larger areas indicating, overall, a better performance.
- **TP count:** True positive number of edges for the same false positive count of $FP=5$ across all methods.

Synthetic data, observations

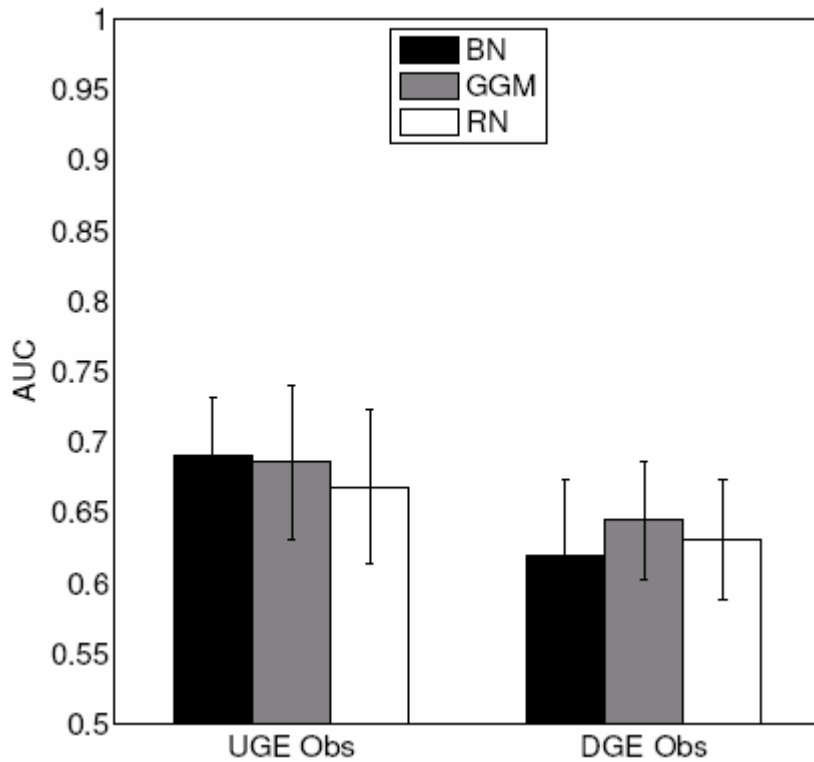


Synthetic data, interventions

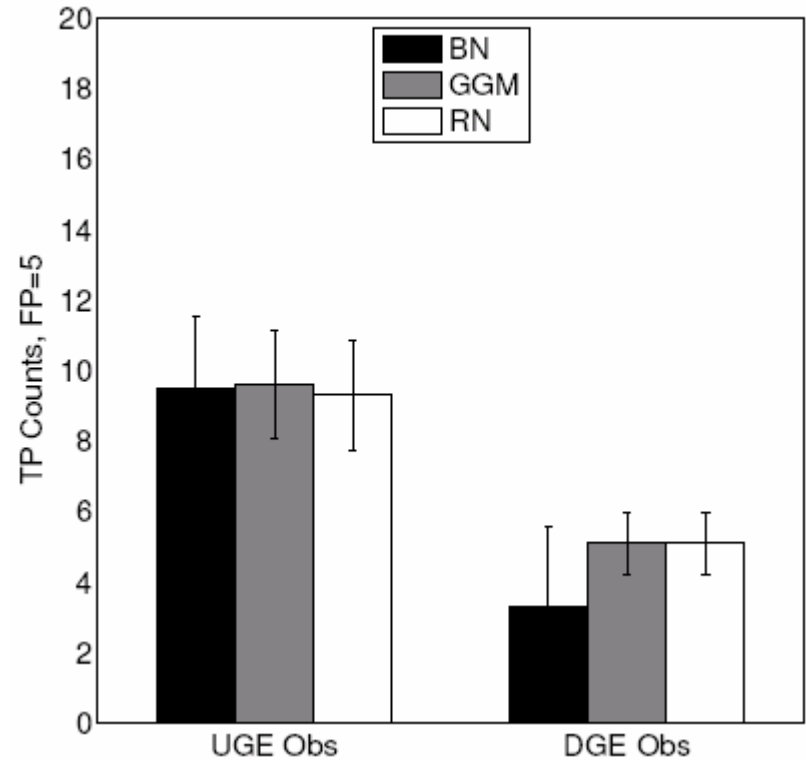


Cytometry data, observations

Real Data – AUC

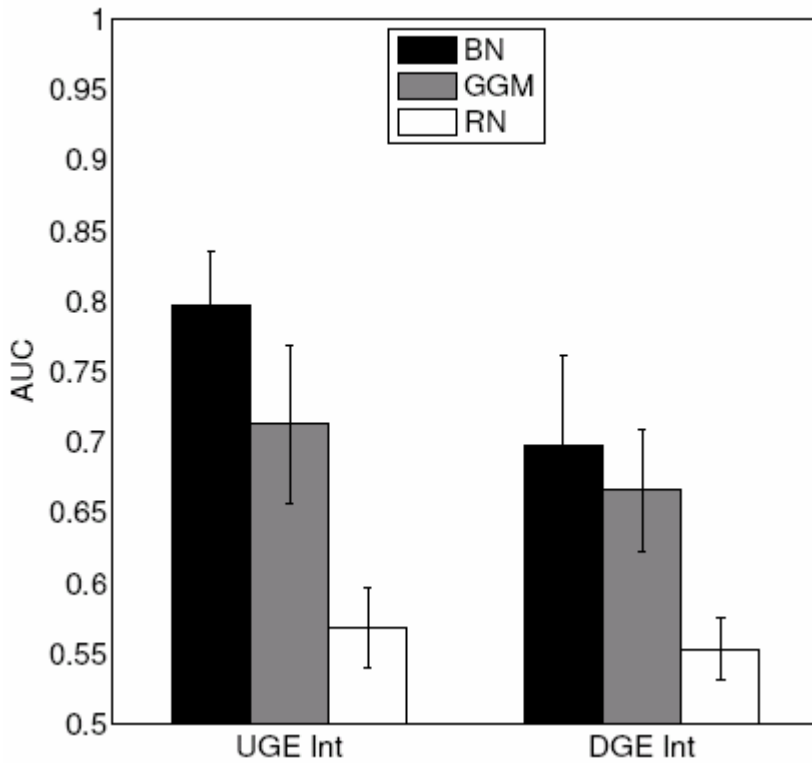


Real Data – TP

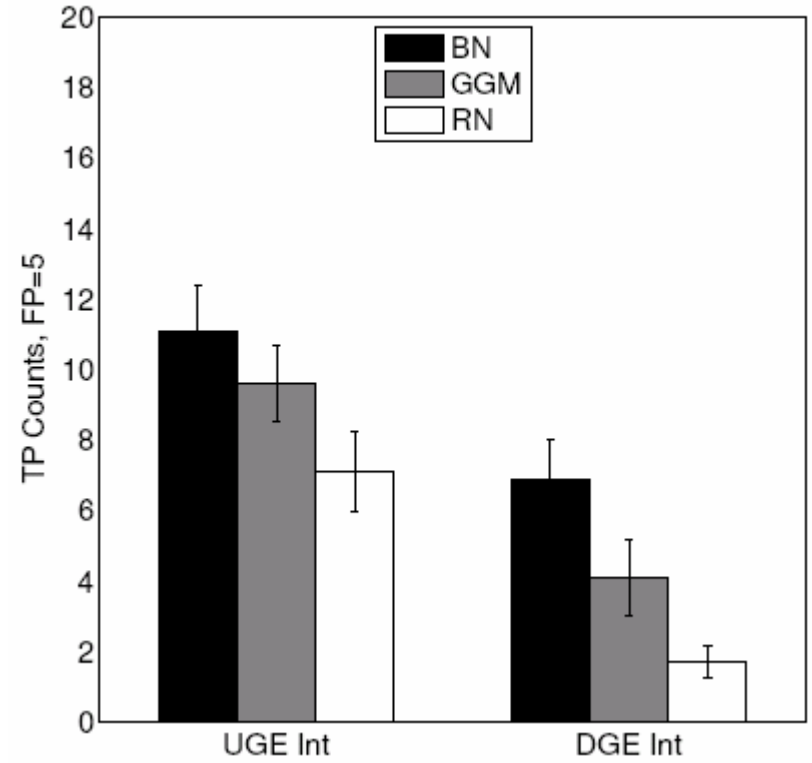


Cytometry data, interventions

Real Data - AUC



Real Data - TP



Conclusions 1

- BNs and GGMs outperform RNs, most notably on Gaussian data.
- No significant difference between BNs and GGMs on observational data.
- For interventional data, BNs clearly outperform GGMs and RNs, especially when taking the edge direction (DGE score) rather than just the skeleton (UGE score) into account.

Conclusions 2

- Performance on synthetic data better than on real data.
- Real data: more **complex**
- Real **interventions** are **not ideal**
- **Errors** in the **gold-standard** network

Disputed structure of the gold-standard network

Regulation of Raf-1 by Direct Feedback Phosphorylation. *Molecular Cell*, Vol. 17, 2005 Dougherty et al

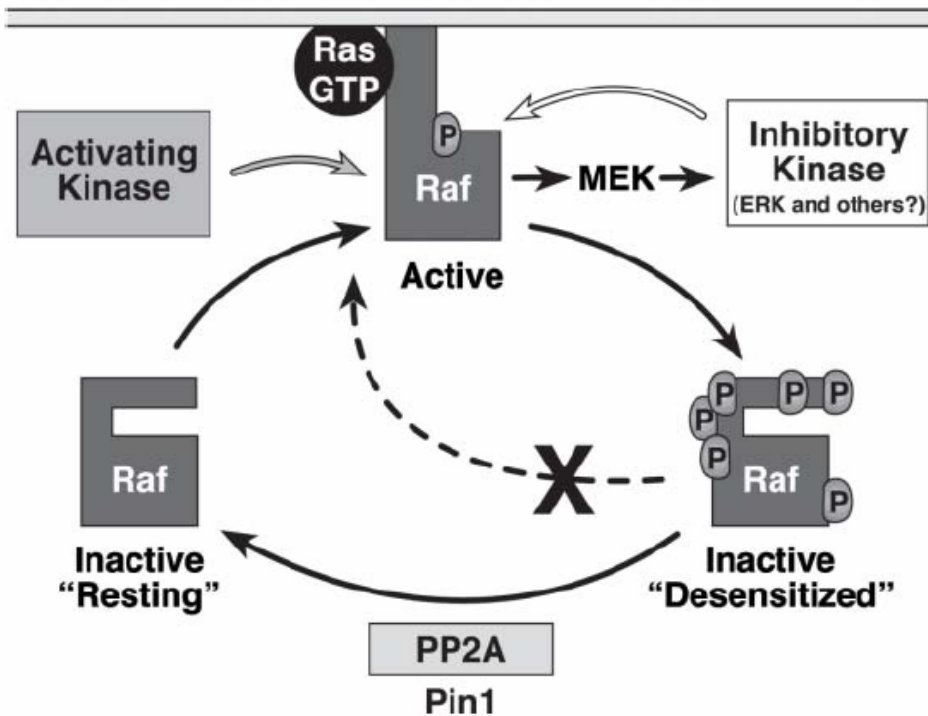


Figure 7. A Model for Raf-1 Regulation by Feedback Phosphorylation

