
# Network Reconstruction

Ulf Leser

# Content

- Network reconstruction
  - Boolean models
  - Correlation-Based Approaches: REVEAL / ARACNE
  - Example
- Quantitative network reconstruction

# Networks

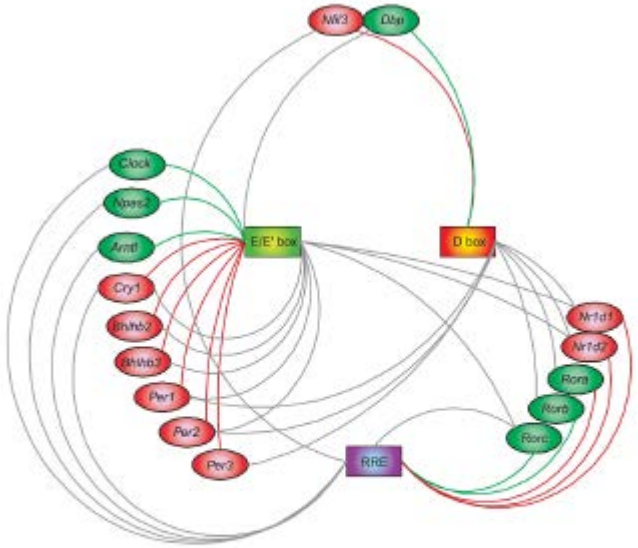
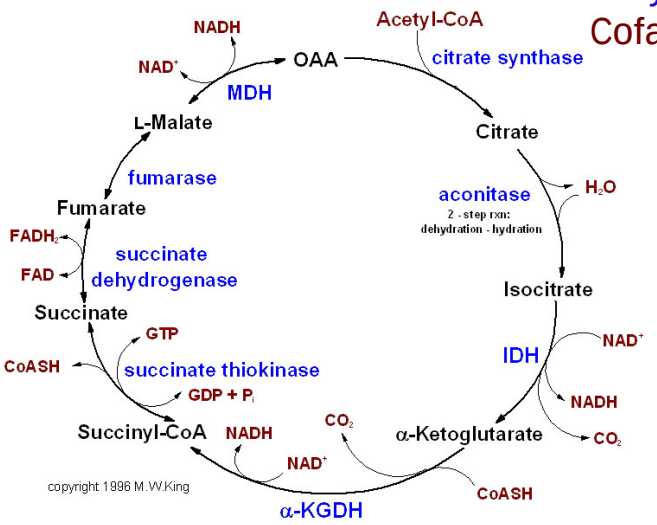



Abbildung 2: Zentrale Gene der zirkadianen Uhr und deren wechselseitiger Einfluss. [UHC+05] (Kästen: Cis-Elemente/Grüne Ovale: Positiv regulierende Gene/Rote Ovale: Negativ regulierende Gene/Regulationsrichtung 1: Von Gen über farbige Kante zu Cis-Element/Regulationsrichtung 2: Von Cis-Element über graue Kante zu Gen)

Chemicals
Enzymes
Cofactors




How do we know? Network reconstruction

# Approaches to Network Reconstruction

- By many, many small-scale experiments
- By mathematical modeling from high-throughput data sets
- By evolutionary inference from model organisms
- By curation from the literature (see first bullet)

# Reconstruction from Indirect High-Throughput Data

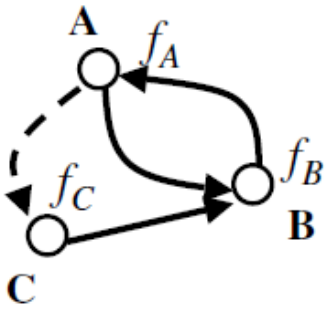
- Network reconstruction, re-engineering, inference, ...
- Idea: Derive network from indirect observations
  - Network: Links and their effect (strength, activation, ...)
    - We usually assume the players (genes, metabolites, ...) to be given
  - Observation: High-throughput measurements
    - Here: Transcriptome, microarrays, RNA-Seq
  - Indirect: We try to infer physical causality by correlation of expression intensities
- Warning: All current methods are highly reductionist

# Reconstruction from Indirect High-Throughput Data

- Quantitative time-resolved network inference: Infer intensities of activities over time
  - Very complicated
- Dynamic networks: Synchronize time and discretize activity
  - Nodes get one of two states: active / inactive
  - Edged determine how states propagate through the network
  - Propagation proceeds in synchronized steps
  - Current states determine future states of connected nodes

# Boolean Networks

- Definition
  *A Boolean Network is a set of nodes V with*
  - *Every node has an associated Boolean state (on/off)*
  - *Every node is labeled with a Boolean function over the states of nodes*
- Visualization
  - We map a BN V into a digraph G=(X,Y) by:
    - X = V
    - Y = { (v,w) | v,w ∈ X and w is part of the boolean function of node v}
  - G has less information than B
    - Boolean formulas cannot be derived from G




$f_A(B) = B$

$f_B(A,C) = A \text{ and } C$

$f_C(A) = \text{not } A$

Boolean Network

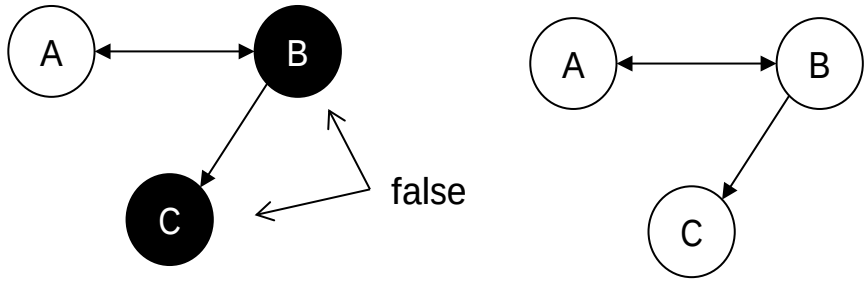
# Boolean Network for Biology

- Vertices = genes
- Boolean formulas: Interplay of other genes necessary to active (reguate) a node
- An edge (v,w) vizualises an effect of v on w
- Simplistic: No cofactors, no cellular context, no binding affinity, no time, no kinetics, ...

# Static Boolean Networks

- Definition
  *A state of a Boolean Network is a labelling of all nodes with TRUE or FALSE.*
  *A state S of a Boolean Network is called consistent, when the state of every node equals the value of its boolean function*
- Remarks
  - Not very interesting – nothing ever changes
  - Not every BN has a consistent state (e.g. $f_A(B)=B$, $f_B(A)=$NOT A)

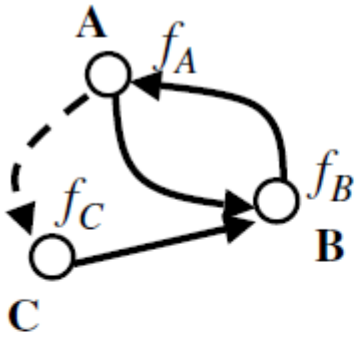
$f_A(B)$ = not B
$f_B(A,B)$ = A and not C
$f_C(B)$ = B



# Network Dynamics

- Definition
  *A Dynamic Boolean Network (DBN) is a Boolean network where every node v is assigned a sequence of states $v_0, v_1, v_2, \ldots$ such that the state of $v_t$ with $t>0$ equals the value of the Boolean function of v applied to the states $w_{t-1}$ of all incoming nodes w of v. The initial states at $t=0$ are arbitrary.*
- Remarks
  - Models the state of every gene over time
  - States at time point t only depend on states at time point t-1
    - No buffering, slow/fast reactions ...
  - Deterministic: Given all states at a time t, any state at any later time point can be uniquely determined

# Example

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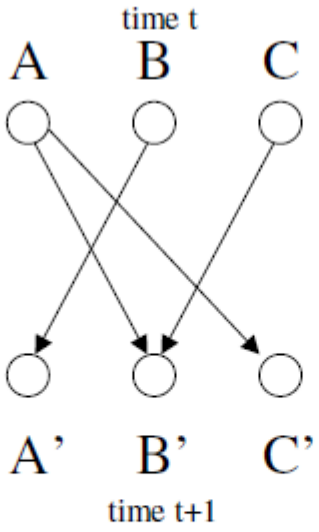
Boolean Network



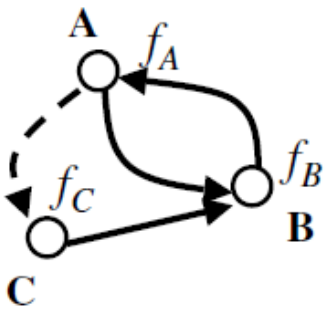

Wiring Diagram

| INPUT | | | OUTPUT | | |
|---|---|---|---|---|---|
| A | B | C | A' | B' | C' |
| 0 | 0 | 0 | 0 | 0 | 1 |
| 0 | 0 | 1 | 0 | 0 | 1 |
| 0 | 1 | 0 | 1 | 0 | 1 |
| 0 | 1 | 1 | 1 | 0 | 1 |
| 1 | 0 | 0 | 0 | 0 | 0 |
| 1 | 0 | 1 | 0 | 1 | 0 |
| 1 | 1 | 0 | 1 | 0 | 0 |
| 1 | 1 | 1 | 1 | 1 | 0 |

Transition table

Source: Filkov, „Modeling Gene Regulation“, 2003

# Example: Changes over TIme




$f_A(B) = B$

$f_B(A, C) = A \text{ and } C$

$f_C(A) = \text{not } A$

Boolean Network

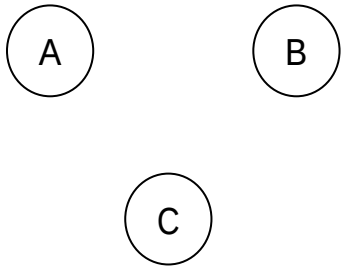
| genes / time | A | B | C |
|---|---|---|---|
| 0 | 1 | 1 | 0 |
| 1 | 1 | 0 | 0 |
| 2 | 0 | 0 | 0 |
| 3 | 0 | 0 | 1 |
| 4 | 0 | 0 | 1 |
| 5 | ... | ... | ... |

# Network Analysis

- Many things can be analyzed using DBN
- For instance, an attractor is a (set of) states towards which the network state converges
  - Point attractor: State which cannot be left any more
  - Cyclic attractor: A series of states which will repeat forever
  - Every DBN must have at least one attractor, as the number of network states is finite – we must "repeat" after at most $2^{|V|}$ steps
  - Number / shape of attractors depend largely on size of network and complexity of Boolean functions
- However, we want to reconstruct networks

# Network Reconstruction

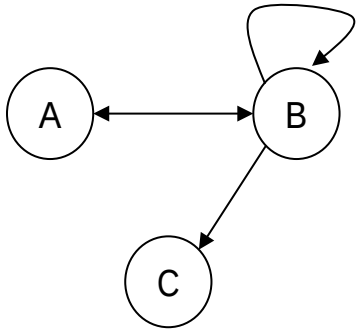
- Assume we know all genes, but not their relationships
- Assume that the states of genes only depends on (the states of) the other genes in the past
- Assume we observe the states of n genes over m time points (a matrix S; the observations)
- Can we re-engineer the Boolean function of every gene given a sequence of states?




S

| genes / time | A | B | C |
|---|---|---|---|
| **0** | 1 | 1 | 0 |
| **1** | 0 | 0 | 1 |
| **2** | 1 | 0 | 1 |
| **3** | 1 | 1 | 0 |
| **4** | 0 | 0 | 1 |
| **5** | ... | ... | ... |

# Example




$f_A(B)$ = not B

$f_B(A,B)$ = A and not B

$f_C(B)$ = B

| genes / time | A | B | C |
|---|---|---|---|
| **0** | 1 | 1 | 0 |
| **1** | 0 | 0 | 1 |
| **2** | 1 | 0 | 0 |
| **3** | 1 | 1 | 0 |
| **4** | 0 | 0 | 1 |
| **5** | ... | ... | ... |

# Formal Problem

- Definition
  *Let $S_t$, $0 \le t \le m$, be the vector of all observed states of all genes at time point t. A DBN G with functions $f_1,\ldots f_n$, $n=|V|$, is called*
  - *consistent with $S_t$ iff $S_t=[f_1(S_{t-1}), f_2(S_{t-1}), \ldots f_n(S_{t-1})]$*
  - *consistent with S iff it is consistent for all $S_t$, $1 \le t \le m$*
- The Boolean network reconstruction problem
  *Given an observation S over a set V, find a DBN G that is consistent with S.*
- Remark
  - Reconstruction means finding the functions $f_1,\ldots f_n$

# Solutions

- Clearly, there are many observations S for which no consistent G exists
  - Recall that DBN are deterministic
  - Imagine $S_t$, $S_{t+1}$ and $S_u$, $S_{u+1}$ with $S_t = S_u$ but $S_{t+1} \neq S_{u+1}$
- Also, there are many observation S for which more than one consistent G exists
- Every time point narrows the options for G – the longer S, the (monotonically) less consistent G's exist

# Optimal Networks

- Definition
  - *For a DBN G, let size(G) be the total number of variables (edges) appearing in the Boolean functions of G*
  - *A DBN G is minimal for observation S, if G is consistent with S and there is no G' which is also consistent with S and size(G')<size(G)*
- Remark
  - Parsimony assumption: Small models are better
  - Thus, the smallest network is the best – functions are as simple as possible, nothing is inferred that is not enforced by the data
  - Not necessarily unique

# Naïve Algorithm

```
N = V;
for k=1…n                          # length of functions
  for every n in N                 # all unexplained nodes
    test all functions f of size k for n on S;
    if f is consistent for n on S
      N := N \ n;                  # n is explained
      Add f to network;
    end if;
  end for;
end for;
```

- Exhaustive naïve algorithm for finding minimal networks
- Very complex (AND, OR, NOT, no paranthesis)
  - k=1: 2n functions
  - k=2: $2*2n*2n=O(n^2)$ functions
  - ...
  - General: $O(2^{2k-1}*n^k)$ functions

# Pros and Cons

- Application (transcriptome data)
  - Perform time-series gene expression experiments
  - Brutally discretize each measurement: Genes are on or off
  - Reconstruct DBN
- Pros: Simple
- Cons
  - Binary values are not capturing reality
  - Nature has no synchronized time or reactions
  - No quantification (“it needs 2*A and one B to regulate C”)
  - Only small networks are solvable
  - No unique solutions
  - ...

# Content

- Network reconstruction
  - Boolean models
  - Correlation-Based Approaches: REVEAL / ARACNE
  - Example
- Quantitative network analysis

# Towards Reality

- There are less complex & more robust algorithms
- REVEAL replaces Boolean functions by mutual information; correlations rather than deterministic switching
- ARACNE is even simpler: Build correlation network and removal some (presumably indirect) correlations

# Foundations

- Definition
  *Let X, Y be two discrete random variables. The mutual information MI(X,Y) is defined as*

$$MI(X,Y) = \sum_{x \in X} \sum_{y \in Y} p(x,y) * log\left(\frac{p(x,y)}{p(x)*p(y)}\right)$$

- Remark
  - Measure the variable's mutual dependency
    - Deviation of observation (p(x,y)) from expectation in case of independence (p(x)*p(y))
    - How much does x determines the state of y (and vice versa)?
    - How important is it to know x to know y (and vice versa)?
- Similar measures: Information gain, pearson correlation, conditional entropy, ...
  - Many are assymetric

# Example

$$MI(X,Y) = \sum_{x \in X} \sum_{y \in Y} p(x,y) * log\left(\frac{p(x,y)}{p(x) * p(y)}\right)$$

| p(x,y) | y=0<br>p(y=0)=0.6 | y=1<br>p(y=1)=0.4 |
|---|---|---|
| x=0; p(x=0)=0.2 | 0,12 | 0,08 |
| x=1; p(x=1)=0.8 | 0,48 | 0,32 |

MI(X,Y)=0

| p(x,y) | y=0<br>p(y=0)=0.6 | y=1<br>p(y=1)=0.4 |
|---|---|---|
| x=0; p(x=0)=0.2 | 0,19 | 0,20 |
| x=1; p(x=1)=0.8 | 0,23 | 0,38 |

MI(X,Y)=0,35

# Two more Facts

- With a little math, we find

$$MI(X,Y) = H(X) - H(X|Y) = H(Y) - H(Y|X)$$

  - H(X): Entropy of X
  - H(X|Y): Conditional entropy of X given Y
- It follows: MI(X,Y) < min(H(X),H(Y))
  - In cace of H(X|Y)=0 or H(Y|X)=0, which means that X (Y) completely determines Y (X)
  - This defines a maximal value for MI(X,Y)
- MI can be extended to sets of three, four, ... variables
  - Like Boolean functions over three, four, ... variables
  - Multivariate mutual information

# Application

- Assume m observation of n genes
  - Can be m time points, m conditions, m samples, m treatments ...
  - REVEAL has no notion of time
- Discretize expression values to 0 or 1 (again)
- Compute for each gene X p(X=0) and p(X=1) is the fraction of observations in which X was 0 / 1
  - Compute for each pair X,Y the probabilities p(X=0, Y=0), ...
  - Compute for each triple X,Y,Z the probabilities ...
  - ...
- Task: Find network such that every node X has the minimal number of incoming edges with maximal mutual information
  - Minimal number of other variables offering maximal explanain

# REVEAL Algorithm

```
N = V;
for k=1…n                          # number of nodes/variables
  for every X in N                 # all unexplained nodes
    find subset T=(Y1,…Yk) with MI(X,Y1,…Yk) = H(X);
    if T exists
      N := N \ X;                  # n is explained
  end for;
end for;
```

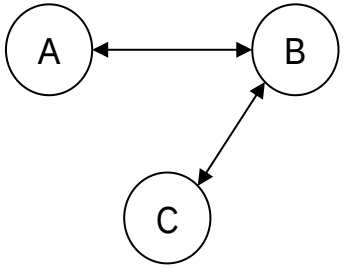
- Very strict: $Y_1,...,Y_k$ must maximally explain X
  - Unrealistic – noise, neglected effects, ...
  - Still very high complexity (“all subsets...”)
- Practical modifications
  - Only require $|MI(X,Y_1,...Y_k) - H(X)| < \varepsilon$
  - Set a maximal k and find best explanation with ≤k edges

# ARACNE

- Fast variation of REVEAL which (a) considers each pair in isolation and (b) gives up model minimality
- Idea
  - Compute mutual information between all pairs of genes
    - This gives a complete network
  - Remove edges where $|MI(X,Y)-H(X)| > \varepsilon$
    - $\varepsilon$ can be estimated from the distribution of MI – created at random?
    - Do not consider composite effects – all Y in isolation
  - Remove certain indirect effects (“data processing inequalities”)

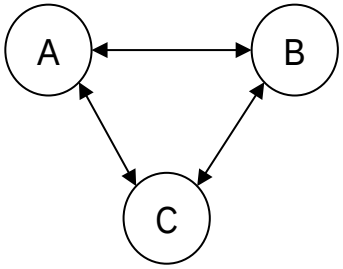
# Data Processing Inequalities

Imagine

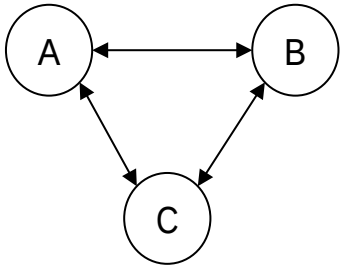



with strong effects of A on B and B on C;

will appears as




But if we find




which edge most probably is an artifact?

- Assumption: If MI(X,Z) ≤ min(MI(X,Y),MI(Y,Z)), then the correlation between X-Z is an indirect effect and removed
- Procedural: In every triangle, remove the smallest edge
  - But in which order should triangles be visited?

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# Reconstructing the Mammalian Clock

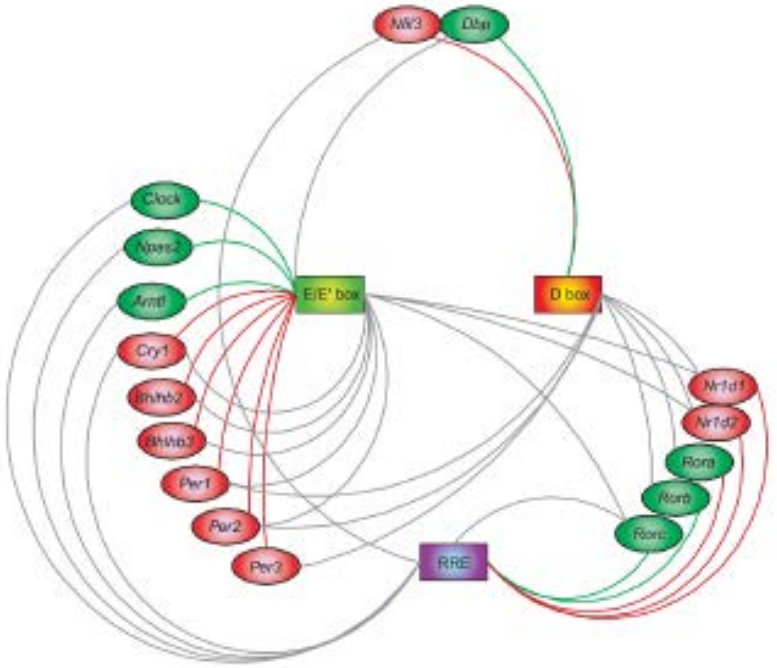



Abbildung 2: Zentrale Gene der zirkadianen Uhr und deren wechselseitiger Einfluss. [UHC+05] (Kästen: Cis-Elemente/Grüne Ovale: Positiv regulierende Gene/Rote Ovale: Negativ regulierende Gene/Regulationsrichtung 1: Von Gen über farbige Kante zu Cis-Element/Regulationsrichtung 2: Von Cis-Element über graue Kante zu Gen)

- DA Sven Lund, 2015
- Data
  - ~630 rather unspecific arrays from GEO
  - Compared to two time-resolved clock-specific experiments
- Reconstruction quality of three algorithms
  - Aracne, Bayes Networks, Time-Delay Aracne

# Results

| Kennzahl | Verfahren | TP | TN | FP | FN | Recall | Precision |
|---|---|---|---|---|---|---|---|
| $\bar{x}$ | Pearson | 53.75 | 20.00 | 41.00 | 21.25 | 0.72 | 0.57 |
| $s$ | Pearson | 4.979 | 8.718 | 8.718 | 4.979 | 0.068 | 0.070 |
| $\bar{x}$ | Bayes | 36.00 | 33.50 | 27.50 | 39.00 | 0.48 | 0.57 |
| $s$ | Bayes | 12.739 | 10.282 | 10.282 | 12.739 | 0.170 | 0.020 |
| $\bar{x}$ | ARACNE | 18.88 | 48.00 | 13.00 | 56.13 | 0.25 | 0.59 |
| $s$ | ARACNE | 5.515 | | | | | |

Averages over all data sets

Averages over all methods

| Kennzahl | Datenquelle | TP | TN | FP | FN | Recall | Precision |
|---|---|---|---|---|---|---|---|
| $\bar{x}$ | GEO | 45.00 | 26.00 | 35.00 | 30.00 | 0.60 | 0.57 |
| $s$ | GEO | 17.550 | 16.480 | 16.480 | 17.550 | 0.235 | 0.034 |
| $\bar{x}$ | Korenčič | 35.67 | 36.22 | 24.78 | 39.33 | 0.48 | 0.60 |
| $s$ | Korenčič | 16.462 | 12.940 | 12.940 | 16.462 | 0.219 | 0.037 |
| $\bar{x}$ | Hogenesch | 30.89 | 36.67 | 24.33 | 44.11 | 0.41 | 0.55 |
| $s$ | Hogenesch | 15.648 | 12.708 | 12.708 | 15.648 | 0.208 | 0.094 |

- Filtering of ARACNE reduces recall a lot, while precision increases only marginally
- Data set size outweighs specificity – reconstruction about as good using many untargeted arrays or using fewer targeted arrays

# Content

- Network reconstruction
- Quantitative network reconstruction

# Networks as Equations

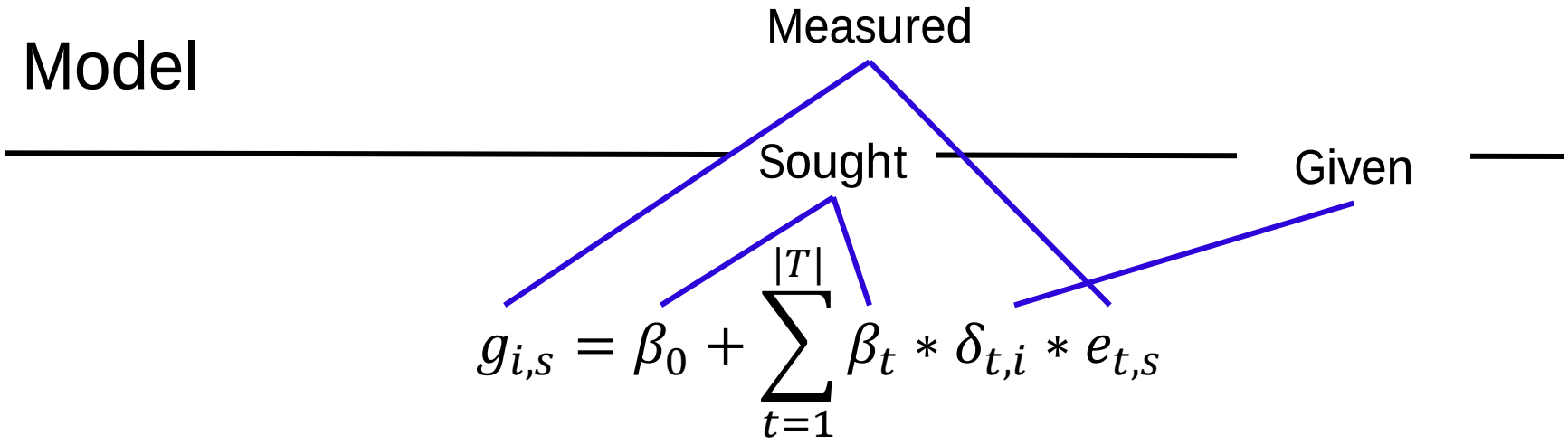
- REVEAL / ARACNE infer relationships based on correlation
- Alternative: Describe states as sets of (linear) equations
  - No discretization
  - Extensibility: Incorporate different types of experiments (“multi omics” – proteome, binding, epigenetic status, ...)
  - Still many limitations: Synchronized time, no kinetics
- We look at one simple approach in between reconstruction and analysis (Schacht et al., 2014)
  - Differentiates between regulators (transcription factors) and regulated entities (genes)
  - Goal: Rank transcription factors by effect strength
    - Which are the most important TFs in this data set?
    - This involves estimating the impact of TF on genes

# Approach

- Assume a network G=(V,E), where V consists of a set of transcription factors T and a set of genes G
  - Transcription factors regulate genes, but not vice versa
    - We ignore that a TF may regulate TFs (even including itself)
  - Each gene g is regulated by all TFs
    - For efficiency, we can also assume this set to be constrained – "potential regulators"
- Measurements: m observations for n nodes (genes / TFs)
- We model the expression values of all genes as linear combinations of the expression values of its regulating TFs

$$g_{i,s} = \beta_0 + \sum_{t=1}^{|T|} \beta_t * \delta_{t,i} * e_{t,s}$$

# Model




- $g_{i,s}$: Expression of gene i in observation s
- $\beta_0$: Fixed additive offset
- $\beta_t$: Global activity parameter for transcription factor t
  - Independent of observation and gene
- $\delta_{t,i}$: Affinity of TF t to gene I
  - E.g. Binding strength to promoter
- $e_{t,s}$: Expression of TF t in observation s

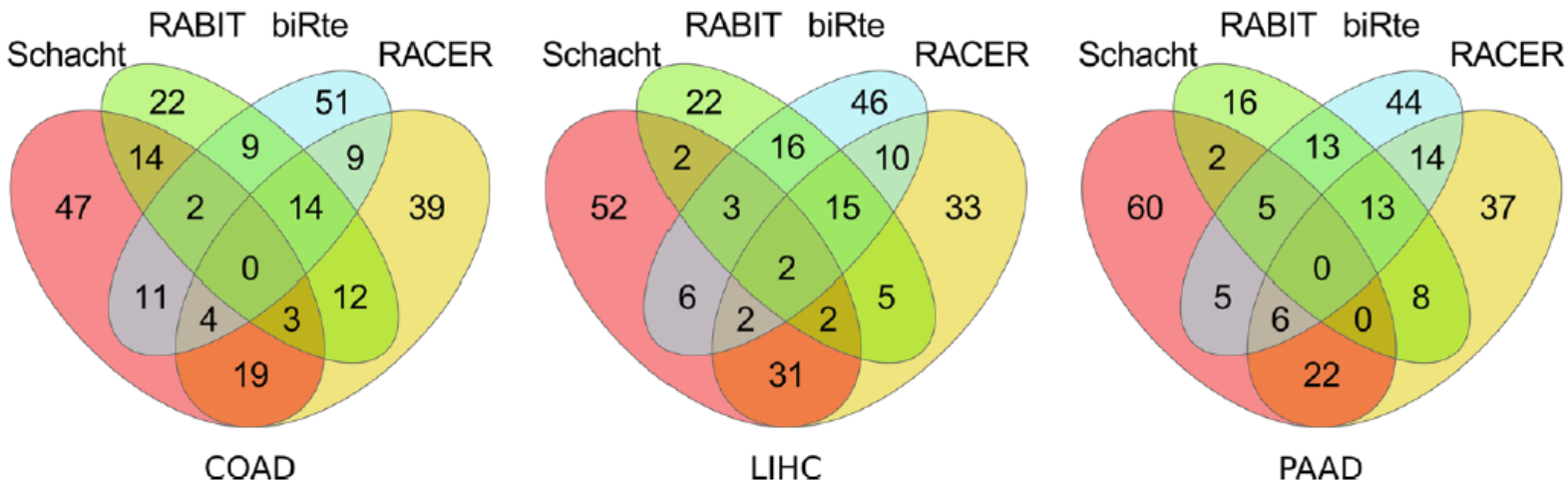
# Optimization

- Typically, these (large) systems cannot be solved exactly
- Instead, minimize the error

$$\left| g_{i,s} - \left( \beta_0 + \sum_{t=1}^{|T|} \beta_t * \delta_{t,i} * e_{t,s} \right) \right| \stackrel{!}{=} min$$

- ... under a set of constraints
- Several solvers available

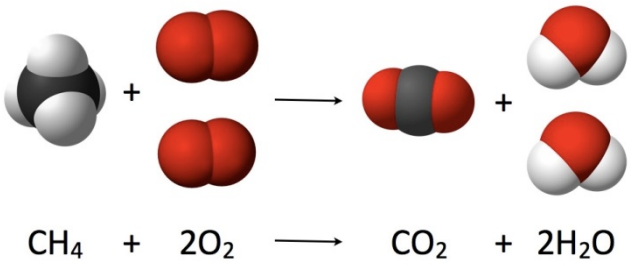
# Comparison (Trescher & Leser, 2018)




- Comparison of different tools shows very little agreement
- Research question essentially open – which method is best? How can we infer regulatory activitiy?

# Many Other Models

- Stoichiometric networks
  - Model the turnover of molecules
    - Especially metabolism
  - Needs to consider enzymatic effects
  - What will a network produce given a certain input?
  - Is a network in flux balance?




- Kinetic networks
  - Takes into account reaction rates: How many in what time
    - No linear relationship
  - Leads to systems of differential equations
  - Can predict system behavior in time under realistic assumptions

# Further Reading


- Liang, S., S. Fuhrman and R. Somogyi (1998). Reveal, a general reverse engineering algorithm for inference of genetic network architectures. Pacific Symposium on Biocomputing., Hawaii, US.
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