Lecture 5: Auto-regulation – a network motif

Chapter 3 of Alon

3.1 Introduction

1. Define a way (based on statistical significance) to detect network motifs
2. Auto-regulation motif
3. Auto-regulation has useful functions: speed-up of response, stabilizer

3.2 Patterns, randomized networks, and network motifs

- To define statistical significance, compare the network to an ensemble of randomized networks
- Patterns that occur in the real network significantly more often than in randomized networks with the same characteristics (number of nodes, number of edges) are called network motifs

- Edges are easily lost in a transcrition network: a mutation that changes a single DNA letter in a promoter can abolish (or create) a binding of a transcription factor and cause the loss or addition of an edge
- See the example in the book (pp 28-29): a change of any DNA letter of the genome can be reached many times very rapidly (within less than a day) in bacterial populations

  => Edges in the network motifs must be constantly selected in order to survive randomization forces in unexpected high amounts
  => Motifs must give some advantages to the organism

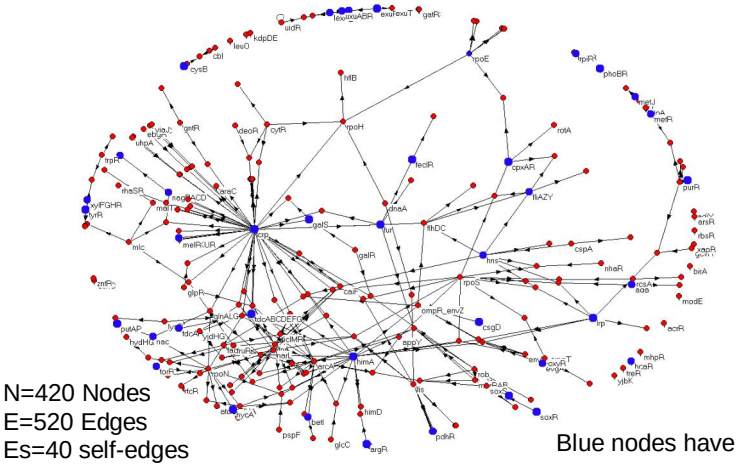
3.2.1 Detecting motifs by comparison to randomized networks

- Erdös-Renyi (ER) model of random graphs: directed edges are assigned at random between pairs of nodes
  - N nodes & E edges

    => There are N^2 possible directed edges (includes the self-edges)

  - In the ER model, the E edges are placed at random in the N^2 possible positions

    => each possible edge is present with probability $p = E/N^2$ (Explain why!)



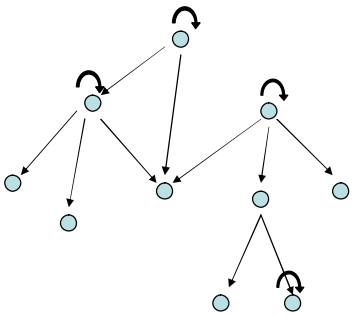
N=420 Nodes
E=520 Edges
Es=40 self-edges

Blue nodes have self-edges

Fig 3.1a. Self-regulating genes in a network of transcription interactions in E. coli. Nodes that correspond to genes (operons) which encode transcription factor proteins that regulate their own promoters (self-regulating genes, represented by self-edges) are shown in blue. This network, which we will use as an example in the coming chapters, has N=420 nodes, E=520 edges and Es=40 self edges.

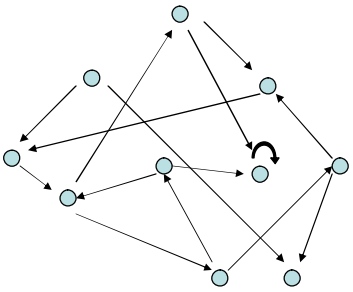
Fig 3.1b

'Real' Network



N=10 nodes
E= 14 edges
Es=4 self-edges

Randomized network
(Erdos – Renyi model)



N=10 nodes
E= 14 edges
Es=1 self-edge

3.3 Auto-regulation - a network motif

- Self-edge: originates and ends at the same node
  - *E. Coli* network: 40 self-edges (Fig 3.1 a)
- Self-edges ↔ auto-regulation
- Negative auto-regulation: repressor proteins that repress their own transcription
  - *E. Coli* network: 34 cases of negative auto-regulation
- Is negative auto-regulation significantly more frequent in the real network than in a random graph with the same number of nodes and edges?

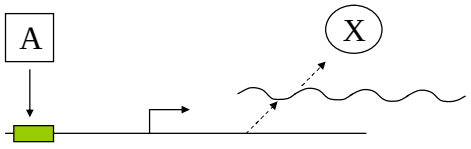


Fig 3.2a: Gene X is simply regulated by A.

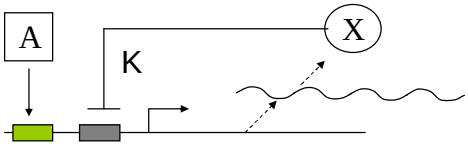


Fig 3.2b: Gene X is **negatively auto-regulated**, and simply regulated by A. Repressor X binds a site in its own promoter and thus acts to repress its own transcription. The symbol --| stands for repression. The repression threshold is K (defined as the concentration of X needed to repress the promoter activity by 50%).

Auto-regulation (cont.)

- The selection probability of a self-edge
  $p_{self} = 1/N$
- Probability of having k self-edges is binomially distributed (throwing a coin E times and getting k heads):
  $$P(k) = \binom{E}{k} p_{self}^{\ k} (1 - p_{self})^{E-k}$$
- Average number < > of self-edges in a random graph (from Poisson approximation of binomial distribution):
  $\langle N_{self} \rangle_{rand} \sim Ep_{self} = E/N$
- Standard deviation of the number of self-edges (Poisson approximation)
  $\sigma_{rand} = (E/N)^{-1/2}$
- **Def. (Z-score):** The Z-score Z(a) of a value a of a random variable x is
  $Z(a) = |a - \text{mean}(x)| / \sigma(x)$
  where σ(x) is the standard deviation of x. Score Z(a) is the deviation from the mean measured in standard deviations.

Example: self-edges of E. coli network are a motif

- *E. coli*'s network (Fig 3.1) has N = 424, E = 519. Then
  $\langle N_{self} \rangle_{rand} \sim E/N \sim 1.2$
  $\sigma_{rand} \sim \sqrt{1.2} \sim 1.1$
  $\langle N_{self} \rangle_{real} = 40$

  => $Z(40) = (40 - 1.2)/1.1 \sim 35$
- 35 standard deviations mark a very high significance
  => self-edges (and also the 34 negatively auto-regulating self-edges for which Z ~ 30) are a network motif

3.4 Negative auto-regulation speeds the response time

- Let protein X be negatively auto-regulated: X → X
- Recall that the dynamics of X is (from the dynamics of X → Y in Lect 4 when X=Y):

$$dX/dt = f(X) - \alpha X$$

- where f(X) = the rate of production of X = the input function for repressive self-regulation of X = the decreasing Hill function

$$f(X) = \frac{\beta}{1+\left(\frac{X}{K}\right)^n}$$

- If X is much smaller than the repression coefficient K, then the production rate of X reaches its maximal value β
- If X is high, then no transcription occurs and hence f(X) ~ 0.

Speed-up of response time (cont.)

- We solve the dynamics using logic approximation where f(X) = 0, if X > K, and f(X) = β if X < K:

$$f(X) = \beta\Theta(X < K)$$

- Let X be initially absent (X=0) and its production starts at t=0. Then

$$dX/dt = \beta - \alpha X \quad \text{while } X<K$$

- At early times we have αX << β. Hence we can neglect degradation α and have

$$X(t) \sim \beta t \quad \text{while } X<K \text{ and } X << \beta/\alpha$$

- However, when X levels reach the self-repression threshold X = K, the production of X stops (small oscillations will occur around X=K if there are delays in the system)
  => X effectively locks itself into a steady-state level equal to the repression coefficient of its own promoter (Fig. 3.3)

$$X_{st} = K$$

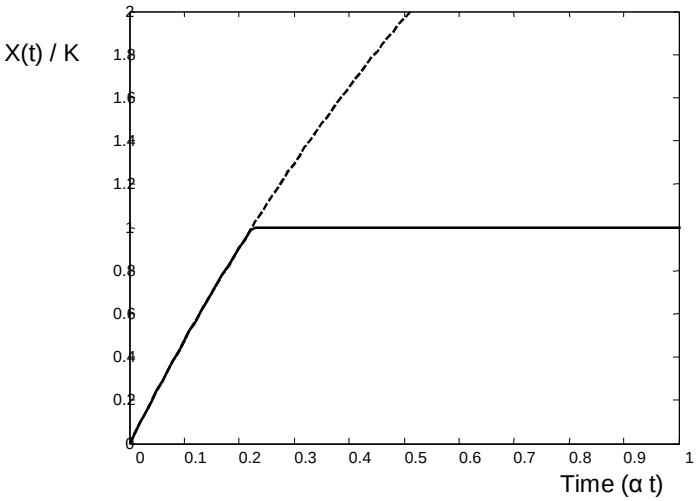


Fig 3.3: Dynamics of a negatively auto-regulated gene product. Production starts at t=0. Full line: Negatively auto-regulated gene with maximal production rate β =5, auto-repression threshold K=1, and degradation/dilution rate α =1. Dashed line: Dynamics of the same gene if auto-regulation is removed, resulting in simple regulation that approaches a higher, unrepressed steady-state $X_{st}=\beta/\alpha=5$.

Speed-up of response time (cont.)

- Response time: $X(T_{1/2}) = X_{st}/2$
- Using linear approximation $X = \beta t$, we obtain

$$T_{1/2}^{(n.a.r.)} = X_{st} / 2\beta = K / 2\beta \quad ((n.a.r.) = \text{negative auto-regulation})$$

- Note: Evolutionary selection can tune parameters β and K independently
  - K modified, for example, by mutations in the binding site of X in the promoter
  - β tuned by mutations in the binding site of RNAp (RNA polymerase) in the promoter

- Response time of simply regulated vs negatively auto-regulated genes?

$$T_{1/2}^{(n.a.r.)} / T_{1/2}^{(n.a.r.)} = (\beta_{simple} / \beta) / 2 \ln 2$$

=> the larger is β, the smaller is the n.a.r. response time as compared with the simple response time (Figs 3.4 & 3.6)

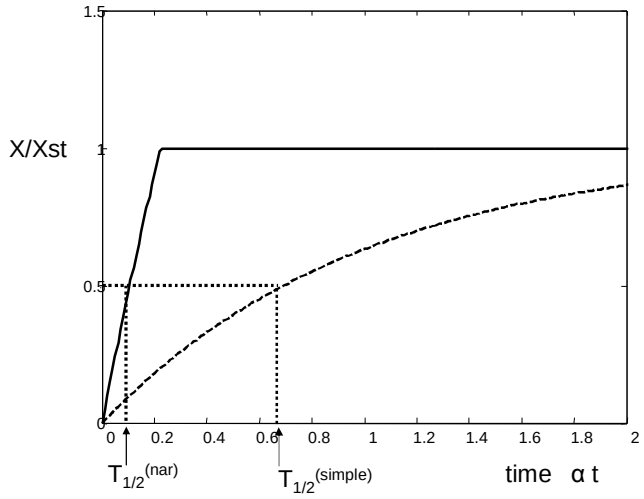


Fig 3.4 Dynamics of negatively auto-regulated gene product (full line) and simply regulated gene product (dashed line) which reach the same steady-state level and have equal degradation/dilution rates α. The response time is the time that the protein level reaches 50% of the steady state, denoted $T_{1/2}^{(nar)}$ and $T_{1/2}^{(simple)}$ for the negatively auto-regulated and simply regulated gene products. The parameters *β=5*, α =1, β_{simple} = *1 were used*.

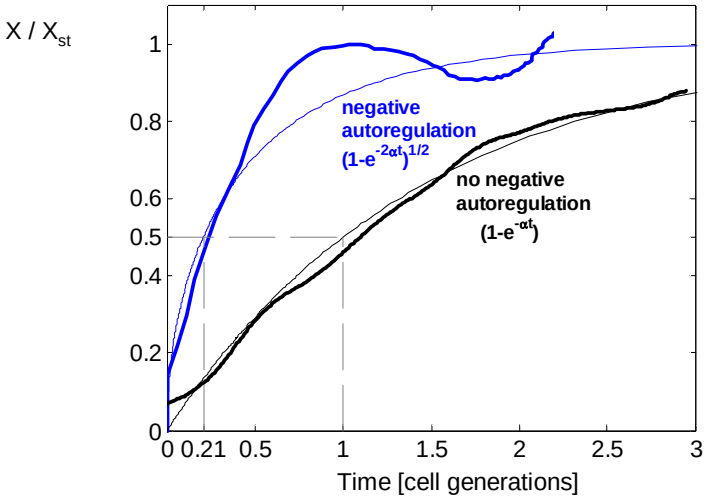


Fig 3.6: Experiment on negatively auto-regulated and simply-regulated genes.The experiment used green-fluorescent protein fused to the TetR repressor as a reporter and automated fluorescence measurements on growing *E. coli* cells. Protein concentration is normalized to its steady-state level. Shown also are the analytical solutions for a simply auto-regulated gene and for a negatively auto-regulated gene with a Hill input function with n=1 in the limit of strong auto-repression (solved exercise 3.1). Source: Rosenfeld, Elowitz, Alon, JMB 323:785 2002

3.5 Negative auto-regulation promotes robustness to fluctuations in production rate

- Simple gene regulation is affected quite strongly by fluctuations in production rate β, as $X_{st} = \beta/\alpha$ and hence a change in β leads to proportional change in X_{st}
- In contrast, negative auto-regulation can buffer such fluctuations, as the steady-state level depends only on the repression threshold of X for its own promoter: $X_{st} = K$
- Positive auto-regulation slows the response time relative to simple regulation (see Fig 3.5). The dynamics are initially slow but with a growing level of X, its production rate increases due to positive autoregulation loop. This results in a concave curve that reaches 50% of its steady-state value at a delay relative to simple regulation.

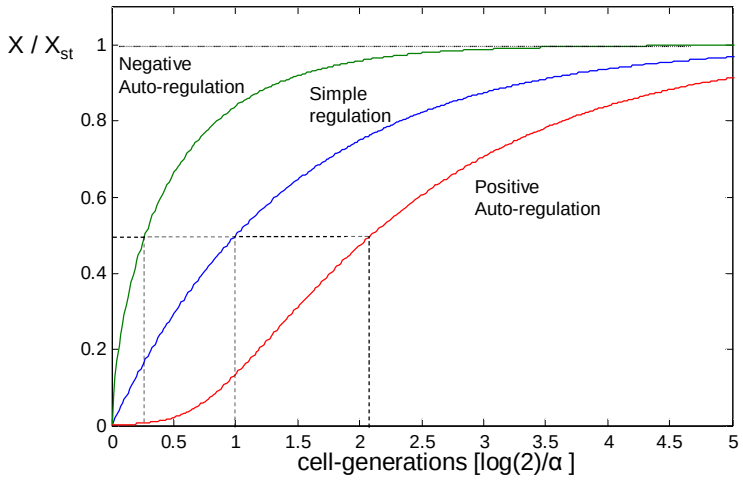


Fig 3.5: Dynamics of negatively auto-regulated gene, a simply regulated gene and a positively auto-regulated gene. The negatively and positively auto-regulated genes have a Hill-input function with Hill coefficient n=1. Shown is protein concentration normalized by its steady-state value, X/X_{st}, following an increase in production rate. Time is in cell-generations, or for actively degraded proteins, log(2)/α, where alpha is the protein degradation/dilution rate. Note that the response-time is $T_{1/2} = \log(2) / \alpha = 1$ for simple regulation, $T_{1/2}$=0.21 for negative auto-regulation, and $T_{1/2}$~2 for positive auto-regulation with the present parameters. The response-time is constructed by the intersect of the dynamics with horizontal line at $X/X_s = 0.5$.