582746 Modelling and Analysis in Bioinformatics

Lecture 4: Network motifs

27.09.2016

Outline

Network motifs

Statistical significance of motifs

Network motifs

- Network motifs are a way to analyze the local structure of a network:
  - What kind of local substructures (motifs, graphlets) does the network have
  - Assessing the statistical significance of these substructures

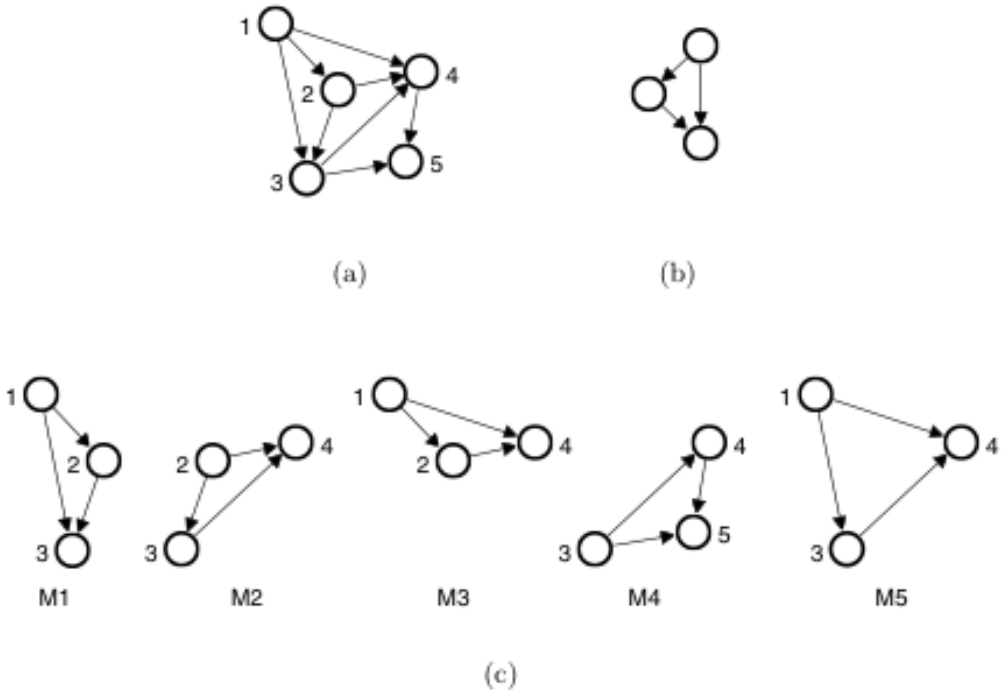


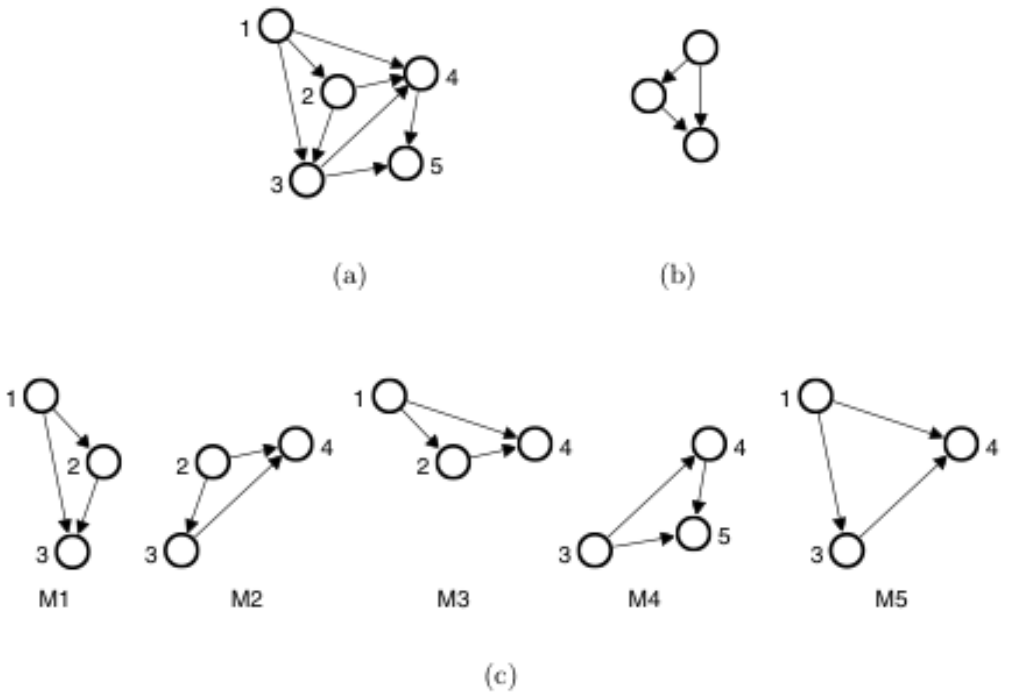
Fig. 2. An example graph (a), a pattern (b) and all different matches of the pattern (c, $M_1 - M_5$). The vertices of the graph and of the matches are numbered consecutively for identification purposes.

What is a motif?

- A *motif* is a statistically overrepresented pattern of local interactions in the network
- Overrepresentation = occurring more frequently than expected by chance
- The rationale is that overrepresentation may denote possible function
  - The motif has emerged several times
  - and it has been conserved in the evolution of the network

What is a motif?

- A *motif* is a small connected subgraph $G' = (V', E')$
- Size of motif is measured either by the number of vertices or the number of edges



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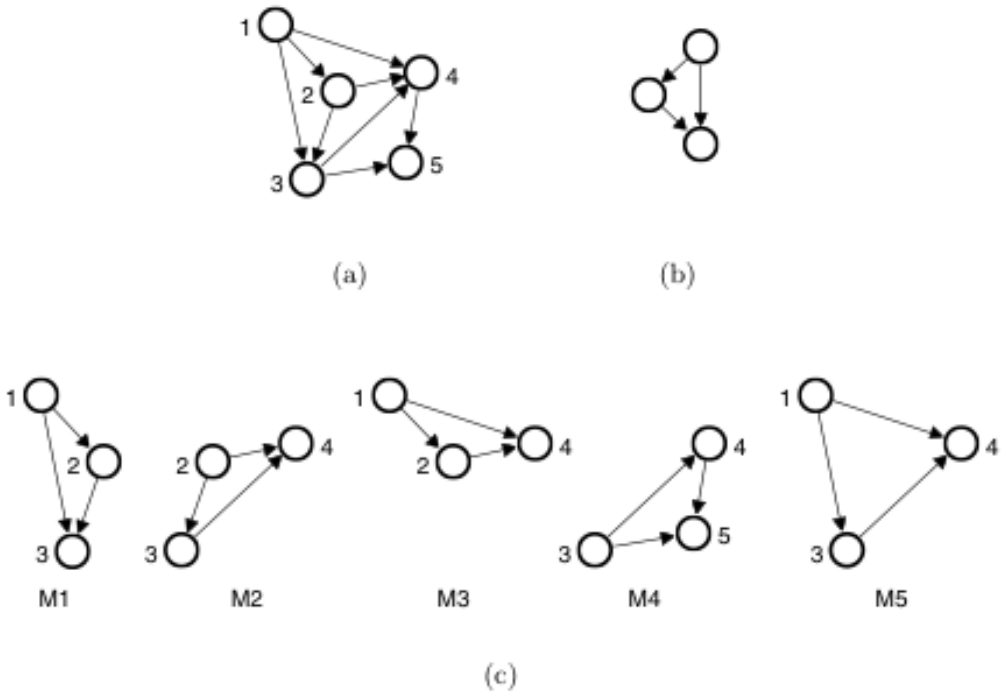
Types of motifs

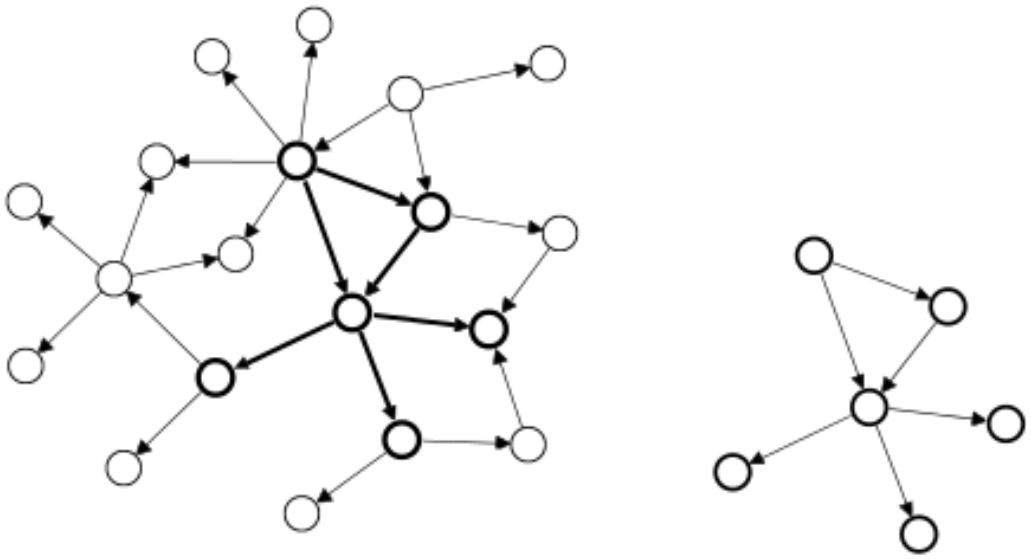
- Motifs can be
  - Directed or undirected
  - Cyclic (loopy) or acyclic

  matching the type of underlying network to be analyzed, e.g.
  - Protein-protein interactions: undirected
  - Gene regulatory interactions: directed, cyclic



Matching motifs

- A *match* of a motif G' in the target graph $G = (V, E)$ is a subgraph $G'' = (V'', E'')$ which is *isomorphic* to motif G'
- Two graphs G' and G'' are isomorphic if there is a bijective mapping between the edge and vertex identities
  - i.e. G' is transformed to G'' by changing the vertex and edge identities

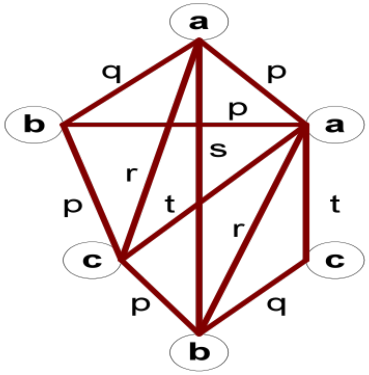


(a) G_T (b) G_P

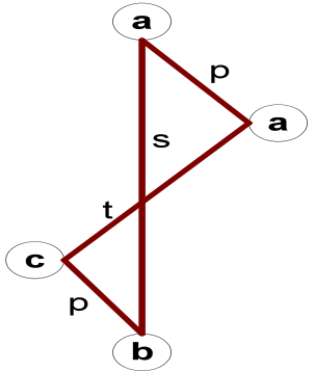
Fig. 1. (a) A graph with a randomly selected subgraph (highlighted with bold lines). This subgraph is isomorphic to the graph G_P shown in (b). The highlighted subgraph in G_T is also a match of G_P in G_T.

Alternative definition: Induced subgraph

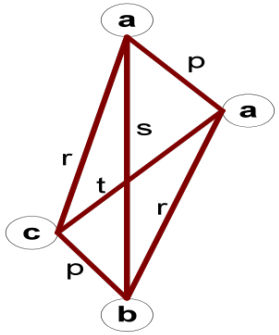
- Isomorphic induced subgraph (graphlet): a subgraph $G'' = (V'', E'')$ in $G = (V, E)$ is accepted as a match only if it contains all edges of the original graph beteeen the nodes in V'': mathematically we require that if $e = (n_i, n_j) \in E$ and $n_i, n_j \in V''$ then $e \in E''$
- Motivation: leaving out interactions from the motif may give false ideas of the biological function



(a) Labeled Graph



(b) Subgraph



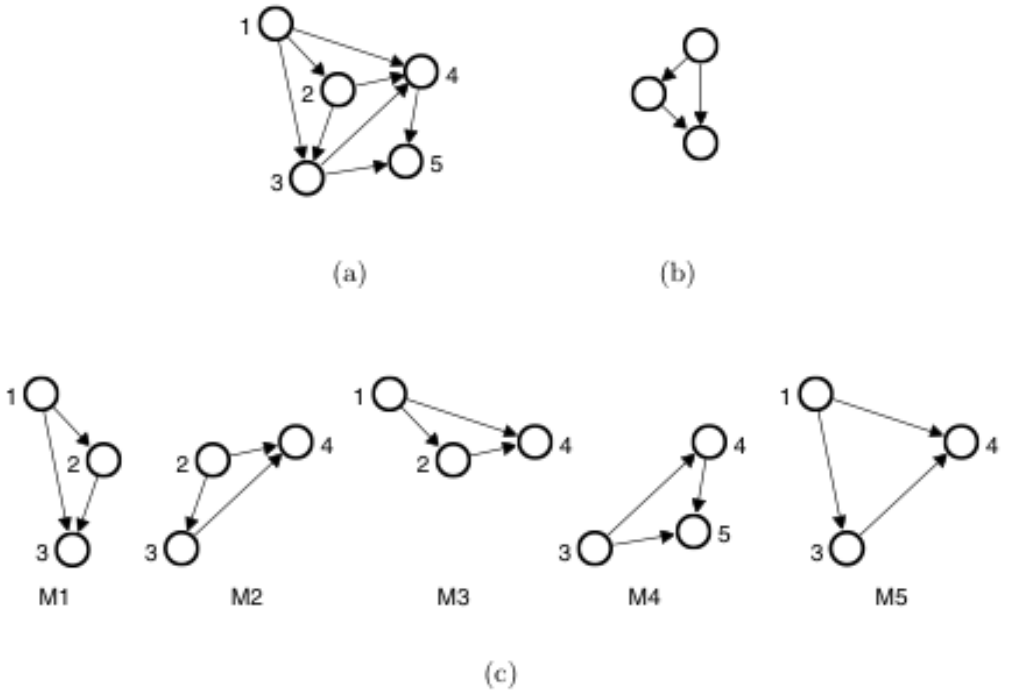
(c) Induced Subgraph

Hardness of isomorphism problems

- The complexity of graph isomorphism is in the 'grey area' of complexity:
  - It belongs to NP class of problems (problems where solution is easy to verify once found)
  - It is not known if graph isomorphism belongs to P class of problems (problems that can be solved efficiently)
  - It is not known if graph isomorphism is NP-complete (problems that are believed to be hard ot solve but easy to verify)
- Subgraph isomorphism, checking if a subgraph G'' that is isomorphic to given graph G' exists in a larger graph G, is known to be NP-complete
- No hope for really fast algorithms for finding motifs.

Motif frequency

- How many times a motif occurs in the network to be analyzed?
- Depends on
  - Definition of a match (subgraph or induced subgraph)
  - Counting schemes for matches



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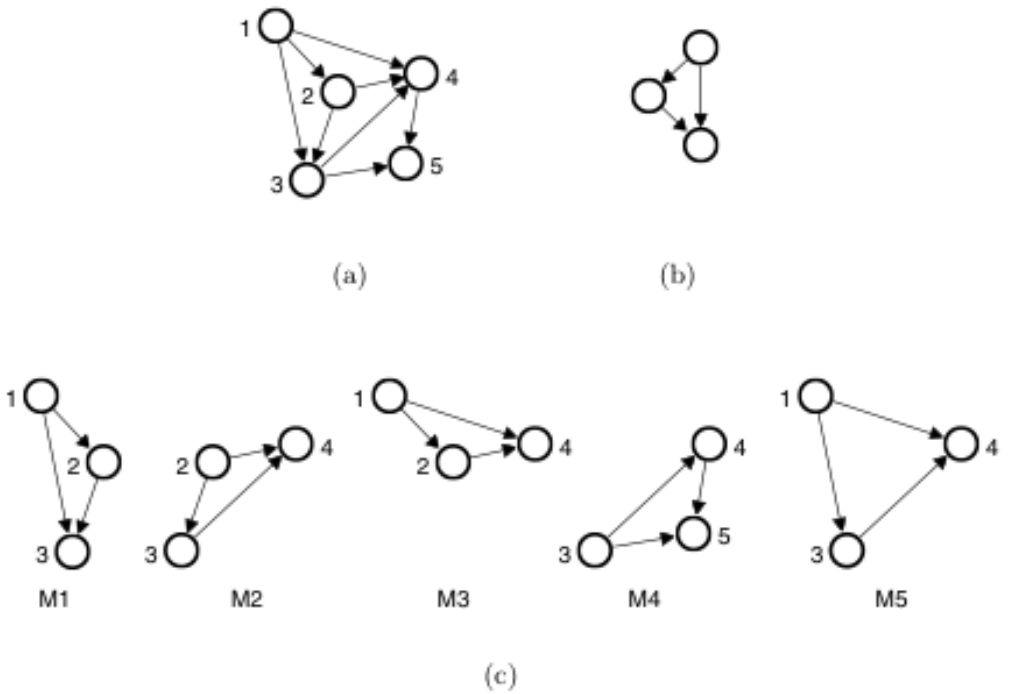
Counting schemes

- Possible schemes for counting matches
  - $\mathcal{F}_1$: Two matches may overlap so that they share vertices and edges
  - $\mathcal{F}_2$:Two matches may overlap so that they share vertices but not edges
  - $\mathcal{F}_3$: Two matches may not overlap, they need to have disjoint sets of vertices



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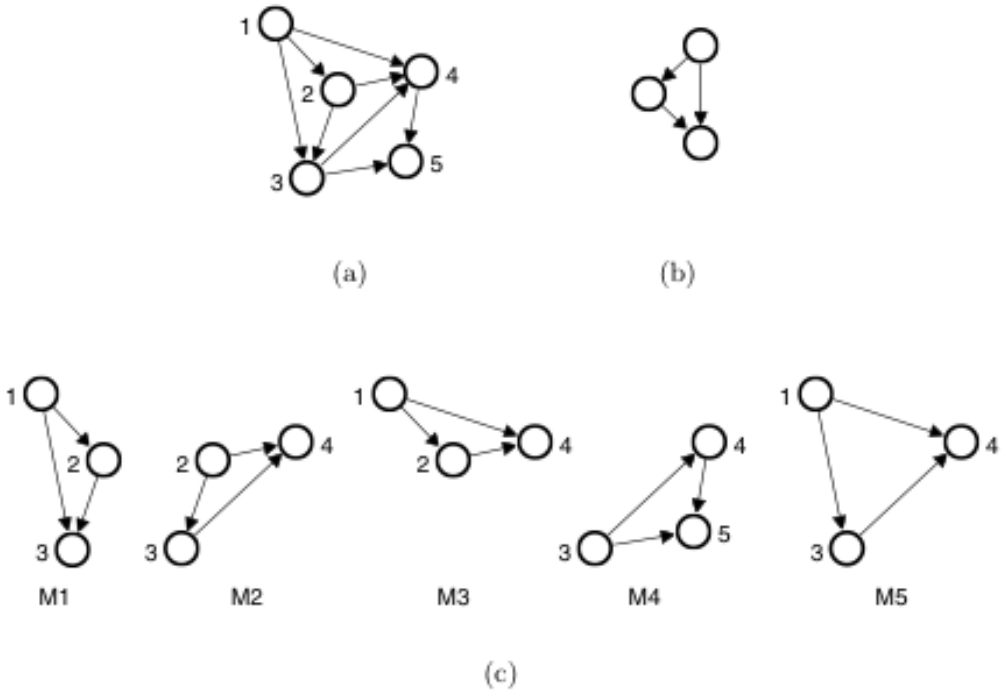
Counting schemes

Concept	Graph elements shared by different matches: Vertices	Edges	Values for the example in Fig. 2: Frequency	Selected matches
$\mathcal{F}_1$	yes	yes	5	$\{M_1, M_2, M_3, M_4, M_5\}$
$\mathcal{F}_2$	yes	no	2	$\{M_1, M_4\}$ or $\{M_3, M_4\}$
$\mathcal{F}^*$	no	yes	–	–
$\mathcal{F}_3$	no	no	1	one of $\{M_1, M_2, M_3, M_4, M_5\}$



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Outline

Network motifs

Statistical significance of motifs

Statistical significance of motifs

- The frequency of a motif in some network does not directly tell us its importance
- Testing for statistical significance is more informative
  - How often we would expect to see this motif by chance in a similar random network
- Need to formulate a *null hypothesis* and check the probability of the motif occurring as frequently under the null hypothesis

Testing fot statistical significance

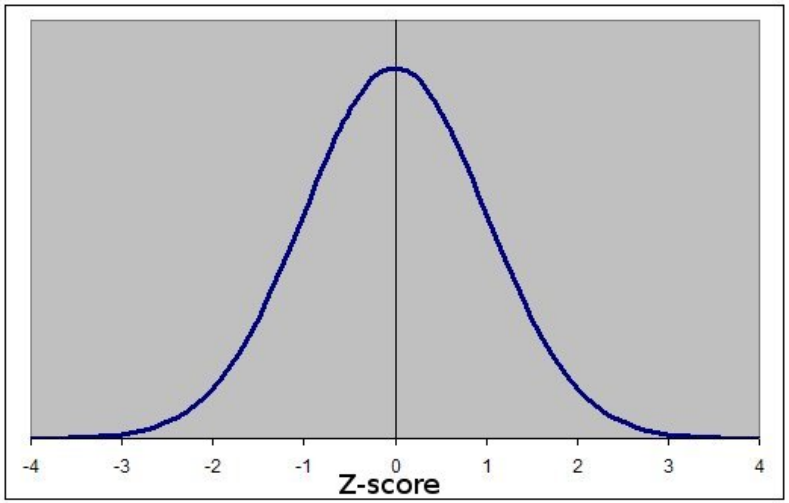
- For a null hypothesis:
- Estimate the probability distribution of the frequency of the motif in random networks
  - Analytically using a network model (e.g. ER networks)
  - By generating an ensemble of random networks
- Measure the statistical significance with Z-score or p-value

Measures of motif significance: Z-score

- Denote by $\mathcal{F}(m)$ the frequency of motif m and by $\overline{\mathcal{F}_r(m)}$ and $\sigma_r(m)$ the average and standard deviation of the motif frequency among the randomized networks.
- Z-score: "how far above the mean of the random networks"

$$Z(m) = \frac{\mathcal{F}(m) - \overline{\mathcal{F}_r(m)}}{\sigma_r(m)}$$

- Z-score above 2.0 is generally considered significant ("two standard deviations")

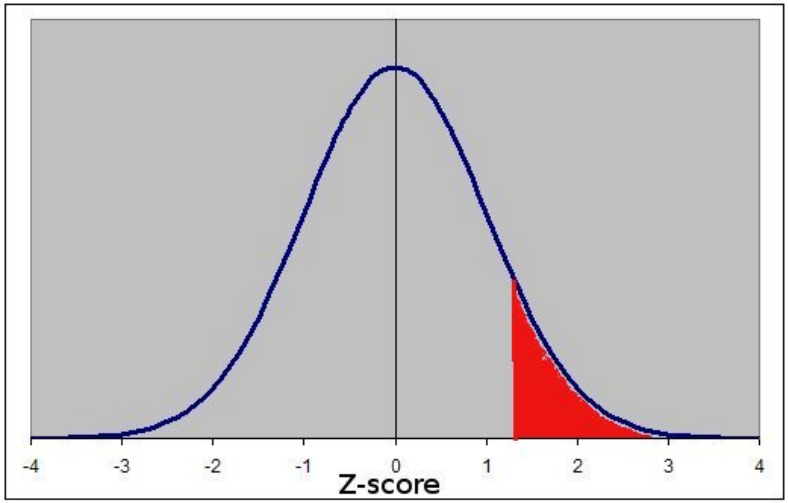


Measures of motif significance: P-value

- P-value: "how often a random network has more motif ocurrences"

$$P(m) = \frac{1}{N} \sum_{r=1}^{N} \mathbf{1}_{\{\mathcal{F}_r(m) \geq \mathcal{F}(m)\}}$$

- $\mathbf{1}_{\{A\}}$ denotes the indicator function, $\mathcal{F}_r(m)$ denotes the motif's frequency in r'th randomized network
- Requires a large number of randomized networks (≈ 1000) to be accurate
  - Estimating the tail of the distribution is harder than estimating its mean (as in Z-score)



Analytical approach using ER networks

- In ER networks an edge is present between two vertices with probability p
- Here we also allow self loops and also these edges are present with probability p
- The ER network should have a similar number of vertices and edges as the real network and so

$$p = \frac{E}{N^2}$$

  where E is the number of edges in the real network and N is the number of vertices in the real network.
- Note that a directed network allowing self loops can have at most N^2 edges.

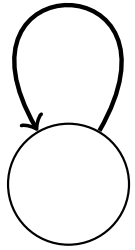
Probability distribution of self loops in ER networks

- The probability of having exactly k self loops is

$$P(k) = \binom{N}{k} p^k (1-p)^{N-k}$$

- The probability distribution is thus binomial with mean:

$$< N_{\text{self}} >= Np = N\frac{E}{N^2} = \frac{E}{N}$$



- and with variance (approximation via Poisson distribution)

$$\sigma_{\text{self}} = \sqrt{\frac{E}{N}}$$

Z-score

- The *E. coli* transcriptional network has 424 vertices and 519 edges (note that this is a different version of the network than what we use in the exercises):

$$
\begin{aligned}
< N_{\text{self}} > &= \frac{E}{N} = \frac{519}{424} = 1.2 \\
\sigma_{\text{self}} &= \sqrt{\frac{E}{N}} = 1.1
\end{aligned}
$$

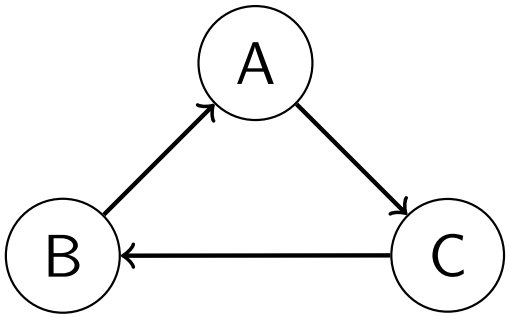
- The real network has 40 self loops:

$$
Z = \frac{N_{\text{self}} - < N_{\text{self}} >}{\sigma_{\text{self}}} = \frac{40 - 1.2}{1.1} = 32
$$

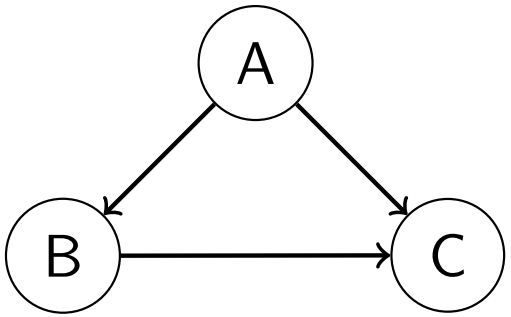
- Z-score is very high and thus the high number of self loops in the *E. coli* transcriptional network is statistically significant

Subgraphs in ER networks

- Consider a pattern graph G with n vertices and g edges
- How often would such a pattern occur in ER networks?
- We will use counting scheme $\mathcal{F}_1$ (vertices and edges can overlap)



Motif 1



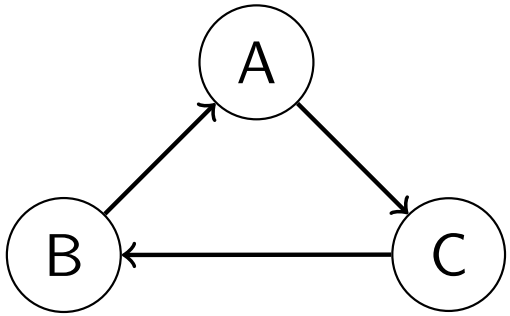
Motif 2

Subgraphs in ER networks

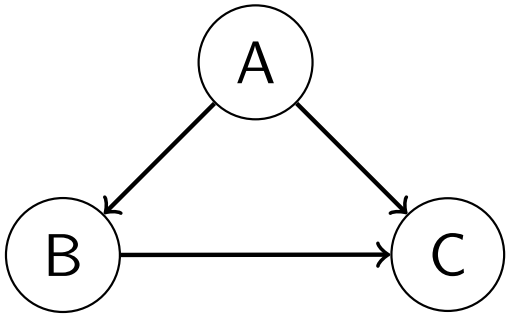
- To generate an instance of this pattern in a random graph, we need to choose n vertices and place the g edges in appropriate places:

$$
\begin{aligned}
< N_G > &= a^{-1} \cdot N \cdot (N-1) \cdot \ldots \cdot (N-n+1) \cdot p^g \\
&\approx a^{-1} N^n p^g
\end{aligned}
$$

where a is the number of permutations of vertex labels of G that give the same graph.



Motif 1
$a = 3$



Motif 2
$a = 1$

Subgraphs in ER networks

- The mean connectivity of a network is

$$\lambda = \frac{E}{N}$$

- and then we get

$$\begin{aligned} < N_G > &\approx a^{-1} N^n p^g \\ &= a^{-1} N^n \left(\frac{E}{N^2} \right)^g \\ &= a^{-1} \lambda^g N^{n-g} \end{aligned}$$

- If we assume that the mean connectivity is constant regardless of the size of the network, then the number of subgraphs scales as

$$< N_G > \sim N^{n-g}$$

Subgraphs in ER networks

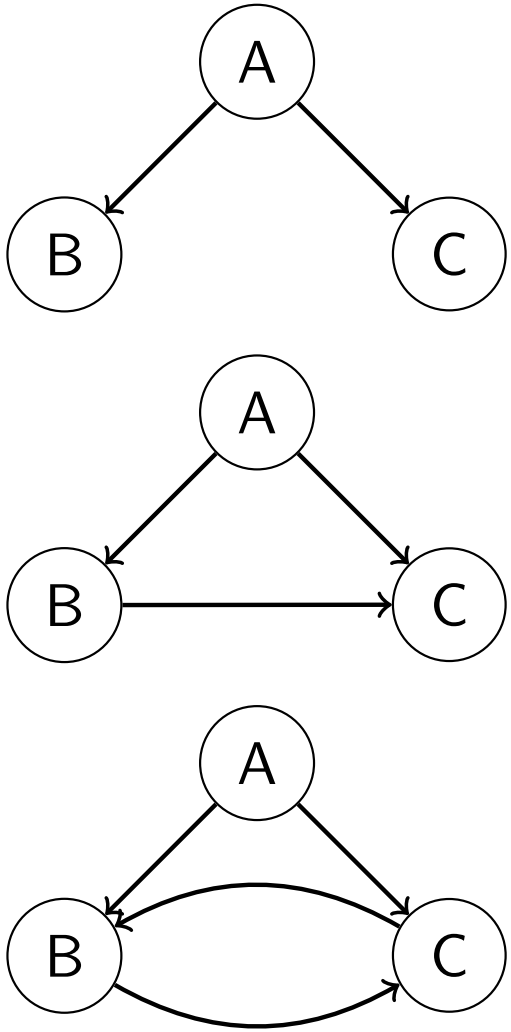
- V-shaped subgraphs (3 nodes, 2 edges) thus scale linearly with the size of the network:

$$< N_{\mathrm{V-shaped}} > \sim N$$

- Number of triangle shaped subgraphs (3 nodes, 3 edges) stays constant:

$$< N_{\mathrm{triangle}} > \sim N^0$$

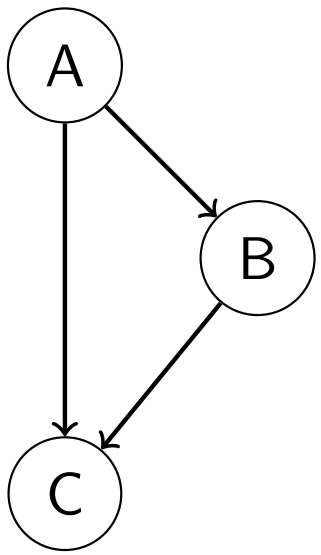
- Subgraphs with 3 nodes and more than 3 edges become rarer when the network gets larger



A subgraph in ER networks and *E. coli* transcriptional regulation network

- The *E. coli* transcriptional regulation network has 424 vertices and 519 edges:

$$< N_{\mathrm{G}} > \approx a^{-1}\lambda^{g}N^{n-g} = \left(\frac{519}{424}\right)^{3} 424^{3-3} = 1.7$$



- The distribution of the motif in ER networks can be approximated by a Poisson distribution and thus the standard deviation is

$$\sigma_{\mathrm{G}} \approx \sqrt{< N_{\mathrm{G}} >} = 1.3$$

- The *E. coli* transcriptional regulation network features 42 instances of the motif and so we get:

$$Z = \frac{42 - 1.7}{1.3} = 31$$

Null hypothesis from random networks

- In traditional hypothesis testing, one typically analytically formulates a probability distribution for the values of the random variable of interest (here frequency of a motif)
- In network analysis, analytically determining a suitable probability distribution may be difficult
- Instead, randomization tests are being used: a large set of random networks of appropriate structure are generated and the average frequency of the motif together with its variance is recorded.
- Computationally demanding process if the networks are large

Randomization algorithm for Null model networks

- Typical method for null model generation is to take the original network being analyzed and make large number of randomized versions of it by modifying the network by a large number of random edit operations
- Commonly used edit operation is to rewire the network locally:
  - Take two edges (A, B) and (C, D) and replace them with edges (A, D) and (C, B)
  - Preserves degree distribution of nodes
  - If the nodes are chosen from a small neighborhood, also keeps average path length close to original

Randomization algorithm for Null model networks

Additional criteria to be preserved can be set, e.g.

- Preserve number of bidirectional edges
- Preserve number of motif of size $n - 1$ when searching for motifs of size n
- ...

As a guideline, the null model should be as close to the original as possible, but randomize the property of interest.

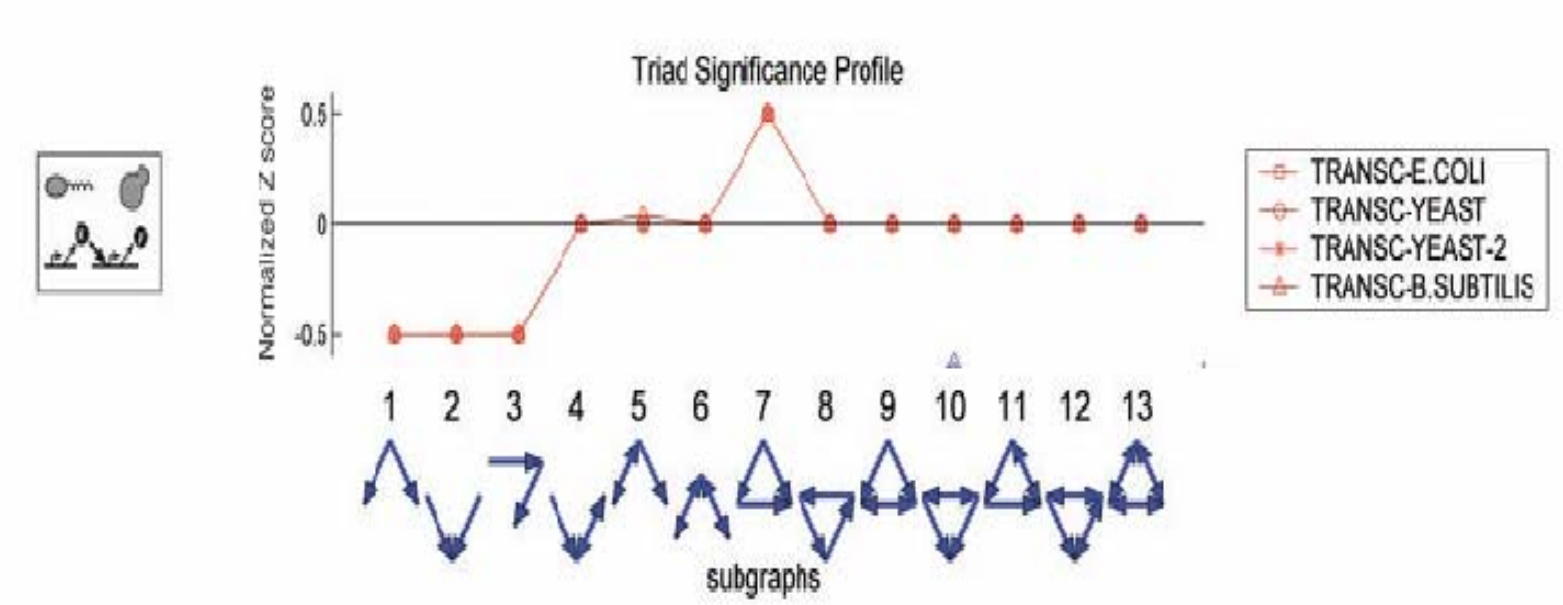
Motif significance profile

- Motif significance profile SP is a vector of normalized Z-scores for a particular set of motifs

$$SP = ((SP(m_1), \ldots, SP(m_2)),$$

where $SP(m) = Z(m_i)/\sqrt{\sum_j Z(m_j)^2}$.

- Motif significance profile allows comparing different size networks in terms of the motifs they contain
- Typically, the set of motifs contains all motifs of particular size



Z. Lin et al. WSEAS Trans. on Comp. 7(6), 2008.

Motifs and antimotifs in PPI networks and internet router network

Pattern	Protein interactions	Internet routers
	Not a motif $C = 0.981$	Not a motif $C = 0.977$
	Motif ($Z = 48$) $C = 0.019$	Motif ($Z = 4600$) $C = 0.023$
	Motif ($Z = 15$) $C = 0.680$	Not a motif $C = 0.931$
	Anti-motif ($Z = -19$) $C = 0.024$	Motif ($Z = 18$) $C = 0.013$
	Anti-motif ($Z = -18$) $C = 0.292$	Anti-motif ($Z = -7$) $C = 0.048$
	Not a motif $C = 0.0013$	Motif ($Z = 356$) $C = 0.004$
	Anti-motif ($Z = -4.5$) $C = 0.0019$	Motif (Z=137) $C = 0.002$
	Not a motif $C = 0.0004$	Motif (Z ND) $C = 0.0005$

Milo et al. Science 298(5594), 2002.

Hardness of motif discovery

Several challenging subproblems:

- Graph isomorphism testing: required to check if two motifs are in fact the same. No polynomial time algorithm is known for this problem.
- Number of motifs: grows exponentially in the size of the motif. Especially with directed motifs grows very fast.
- Number of matches: theoretically the worst case number of potential matches is $O(|E_t|^{|E_m|})$ where E_t and E_m are the number of edges in the target and motif, respectively.
- Size of analyzed networks affects the above steps via the number of different patterns and matches that can be found.
- Calculation of statistical significance via randomization calls for generation and motif discovery from a large number of networks, multiplying the computation time of all the above points.

Study Group on Thursday

- Group 1: Students whose first name has exactly 5 characters
  - F. Schreiber and H. Schwbbermeyer: Frequency concepts and pattern detection for the analysis of motifs in networks. Trans. on Comput. Syst. Biol: III, pp. 89–104, 2005.
  - Concentrate on section 4.
- Group 2: Students whose first name does not have exactly 5 characters
  - N. Kashtan, S. Itzkovitz, R. Milo and U. Alon: Efficient sampling algorithm for estimating subgraph concentrations and detecting network motifs. Bioinformatics 20(11):1746–1758, 2004.
  - Concentrate on the Methods section.