

Detecting Network Motifs by Local Concentration

by

Etienne Birmelé



Research Report No. 23
March 2009

STATISTICS FOR SYSTEMS BIOLOGY GROUP
Jouy-en-Josas/Paris/Evry, France
<http://ssbgroup.fr>

Abstract: Studying the topology of so-called *real networks*, that is networks obtained from sociological or biological data for instance, has become a major field of interest in the last decade. One way to deal with it is to consider that networks are built from small functional units called *motifs*, which can be found by looking for small subgraphs whose numbers of occurrences in the whole network of interest are surprisingly high. In this paper, we propose to define motifs through a local overrepresentation in the network and develop a statistic which allows us to detect them limiting the number of false positives and without time-consuming simulations. We apply it to the Yeast gene interaction data and show that the known biologically relevant motifs are found again and that our method gives some more information than the existing ones.

1 Introduction

Recent work indicates that biological networks show recurrent small patterns, called *network motifs* [17]. They can be thought of as small units of given function from which the networks are built. For instance, Alon [2] describes the regulation role in transcriptional networks of a pattern of 3 vertices called the feed-forward loop.

In order to reach a better understanding of the structure of biological networks, it is then quite natural to ask which are the small patterns that are over-represented in those networks. Many attempts were made to answer that question. Some of them [17] simulate the distribution of the number of occurrences of a subgraph using a huge number of random networks with the same degree distribution as the observed one, but they seem not to be tractable for motifs on more than four vertices. Others [12, 20] rely on the calculation of a Z-score. Nevertheless, the distribution of the number of occurrences of small subgraphs is more heavy-tailed than a gaussian distribution and therefore those methods may lead to false positives.

To avoid simulations, one has to define a probabilistic model of random graph generation and to compute the p-value of the observed number of subgraphs in that model.

That approach leads to a first step which is the choice of a random model to describe biological networks and which is simple enough to deal with combinatorics on it. The simplest random graph model is the Erdős-Rényi model [9], that is a graph on a fixed number of vertices whose edges are sampled as independent identical Bernoulli variables.

Nevertheless, such random graphs do not show the heterogeneity of biological networks. Many more realistic models were therefore developed [3, 4, 18] and in particular, mixture models were studied in [7, 14]. The main advantage from the latter is to give rise to heterogeneous graphs but which still depend on independent Bernoulli trials.

Picard et al. [19] propose to take a mixture model as the null model. As they can't compute the law of the number of occurrences of small subgraphs under that model, they propose to fit the best possible Polya-Aeppli distribution to the

subgraph distribution and to take the p-value of that approximate distribution.

As pointed out in [17], another issue is that a small graph can appear as over-represented because it contains an over-represented subgraph, which is in fact the biological relevant structure. Moreover, Dobrin et al. [8] show that the motifs in the yeast transcriptional regulatory network aggregate. For both reasons, we will consider a new definition of a motif: given a small graph \mathbf{m} and a fixed occurrence in the network of one of its subgraphs \mathbf{m}' , we will look for an over-representation of the number of occurrences of \mathbf{m} extending the given occurrence of \mathbf{m}' . In other words, a motif will be defined by a local over-representation rather than by a global one.

We propose to tackle the problem of the computation of the p-value by considering a model with independent edges, each one appearing with a given connectivity probability. Moreover, to avoid a bias due to the use of an approximate distribution, we will look for an upper bound of the real p-value. That conservative approach in motif detection induces a better limitation of the number of false positives.

To do so, we will use concentration inequalities. This term is used to characterize inequalities which bound the probability of a random variable to be far away from its expectation. The most widely known is Chebychev's inequality which states that, for every random variable X and positive real t ,

$$\mathbb{P}(|X - \mathbb{E}X| > t) \leq \frac{\text{var}(X)}{t^2}$$

Nevertheless, an abundant literature exists to show that under some conditions, the bound of $\mathbb{P}(X - \mathbb{E}X > t)$ decreases in fact exponentially with respect to t . The term *concentration inequalities* expresses that X is heavily concentrated around its mean value. Surveys and recent work on such inequalities can be found for instance in [6, 13, 16]. We will use such an inequality to show that the number of motifs aggregating on a given submotif is highly concentrated.

In the following, we will consider directed networks. A network is then a graph $G = (V, \vec{E})$ of vertex set V and edge set \vec{E} . We suppose that there are no multiple edges in the same direction but opposite edges between two vertices and self-loops are allowed.

For any vertex set $U \subset V$, we denote by $G[U]$ the *induced* subgraph of G on the set U , that is the graph of vertex set U where each edge is present if and only if it is present in G . If U is an ordered set, the vertices of $G[U]$ are ordered in the same way.

The paper is organized as follows: Sections 2 and 3 respectively detail the definition of a motif and the random graph model which will be used throughout the paper. The main result is given and proved in section 4. Finally, in Section 5, we apply our method to the Yeast gene interaction network. We show that the known over-represented motifs of size 3 and 4 of that network are found again

and that the knowledge of the submotifs with respect to which they are over-represented allows to sort non-relevant motifs and to distinguish the role of the vertices in the relevant ones. Moreover, our method doesn't need any simulation and is therefore significantly faster than the existing ones.

2 Network motifs and submotifs

A *network motif* \mathbf{m} of size k is a directed graph on k vertices, with possible loops and opposite edges, that is that the two edges \overrightarrow{uv} and \overleftarrow{vu} between two vertices u and v may occur simultaneously.

An *ordered motif* is a network motif whose vertices are ordered. Let \mathbf{m}_0 and \mathbf{m}_1 be two ordered motifs of size k and let (u_1, \dots, u_k) and (v_1, \dots, v_k) be their respective ordered vertex sets. We will say that \mathbf{m}_0 and \mathbf{m}_1 are *isomorphic as ordered motifs* and we will note $\mathbf{m}_1 \sim_o \mathbf{m}_2$ if, for every pair of integers (i, j) , $\overrightarrow{u_i u_j}$ is an edge of \mathbf{m}_0 if and only if $\overrightarrow{v_i v_j}$ is an edge of \mathbf{m}_1 .

Figure 1 shows two ordered motifs that are not isomorphic as ordered motifs, despite of the isomorphism of the underlying non-ordered motifs.

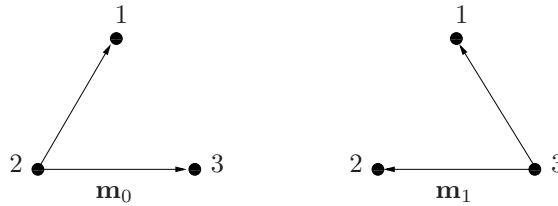


Figure 1: Isomorphic motifs that are not isomorphic as ordered motifs. The numbers represent here the orders of the vertices.

The ordering of the motifs and the notion of ordered isomorphism are introduced for convenience in the latter formulas, but they do not influence the over-representation of a motif as we shall see below.

Indeed, let \mathbf{m} be a motif on k vertices and denote by $N(\mathbf{m})$ the number of distinct occurrences of the motif in G . Let $aut(\mathbf{m})$ be the number of automorphisms of \mathbf{m} . Taking one ordering of the motif, $aut(\mathbf{m})$ can be seen as the number of orderings of the vertices which give rise to ordered motifs isomorphic to the first one. For example, if \mathbf{m} is the motif on three vertices with two edges starting from the same vertex, the ordered motif \mathbf{m}_0 in Figure 1 gives an ordering of the motif \mathbf{m} . The only other isomorphic ordering is obtained by exchanging the labels 1 and 3, so $aut(\mathbf{m}) = 2$.

Let us order the vertices of \mathbf{m} to obtain an ordered motif \mathbf{m}_o . Then, describing all the ordered sets of k vertices in G and looking for an ordered isomorphism with \mathbf{m}_o , each occurrence of \mathbf{m} is counted $aut(\mathbf{m})$ times and finally

$$\text{aut}(\mathbf{m})N(\mathbf{m}) = \sum_{(i_1, \dots, i_k)} \mathbb{I}_{G[(i_1, \dots, i_k)] \sim_o \mathbf{m}_o} \quad (1)$$

for any choice of an ordered version \mathbf{m}_0 of \mathbf{m} .

Let \mathbf{m} be a motif on k vertices. A *submotif* of \mathbf{m} is then a subgraph of \mathbf{m} . In this article, we only consider the submotifs obtained by suppressing one vertex in \mathbf{m} . That approach gives rise to a partition of the vertices of \mathbf{m} into *deletion classes*, two vertices r and s of \mathbf{m} being in the same deletion class if and only if they play the same topological role in \mathbf{m} . In other words, r and s are in the same class if, given an ordering \mathbf{m}_0 of \mathbf{m} , the ordered motif obtained by interverting the labels of r and s is isomorphic to \mathbf{m}_0 .

If two vertices are in the same deletion class, the submotifs obtained by deleting r and s are isomorphic. Nevertheless, the opposite may not be true. Indeed, consider the feed-forward loop motif shown in Table 1. Deleting any of the three vertices leads to a single edge but all the vertices are in different deletion classes as they are not topologically equivalent (they have for instance different outdegrees).

3 The random graph model

Concentration inequalities are powerful tools but mostly apply on functions of *independent* random variables. Moreover, they make use of the expectation of the function of interest. Therefore, we have to consider random graph models satisfying the following conditions, which will also turn to be sufficient for our purpose:

- (i) All the edges are drawn independently.
- (ii) The expectation of any motif count is tractable.

Let us consider the random graph model on n vertices defined by a connectivity matrix $C = (c_{ij})_{1 \leq i, j \leq n}$ and such that all the edges are sampled independently under Bernoulli laws:

$$X_{ij} = \mathbb{I}_{\vec{ij} \in \vec{E}} \sim \mathcal{B}(c_{ij}).$$

It is straightforward under that model to derive an expression for the mean number of occurrences of a given ordered motif \mathbf{m}_0 of size k .

Indeed, let $1, \dots, k$ be the vertices of \mathbf{m}_0 and denote by e_{rs} the indicator function for the edge from vertex r to vertex s in \mathbf{m} . Let (i_1, \dots, i_k) be any ordered list of k distinct vertices of G . Then

$$\mathbb{P}(G[(i_1, \dots, i_k)] \sim_o \mathbf{m}_0) = \prod_{1 \leq r, s \leq k} c_{i_r i_s}^{e_{rs}} (1 - c_{i_r i_s})^{1 - e_{rs}}$$

Therefore, by Equation (1), one has

$$\mathbb{E}(N(\mathbf{m})) = \frac{1}{\text{aut}(\mathbf{m})} \sum_{(i_1, \dots, i_k)} \prod_{1 \leq r, s \leq k} c_{i_r i_s}^{e_{rs}} (1 - c_{i_r i_s})^{1 - e_{rs}},$$

That model has no practical interest as it has as many parameters as possible edges but it is a very general framework in which our theory holds. In practice, we will be able to apply our concentration results on all special cases of that model, which include the Erdős-Rényi model [9] and the Expected Degree Model of Matias et al. [15]. The general model introduced by Bollobás et al. [5] can also be used when restricted to the case of a fixed sequence of vertices. The last interesting family of models which fits in that frame is the one of Mixture Models [1, 7, 10] where the classes of the vertices are fixed.

4 Concentration of Network Motifs

Let \mathbf{m} be an ordered motif on k vertices and \mathbf{m}' the sub-motif of \mathbf{m} on $k - 1$ vertices obtained by deleting a vertex s from a given deletion class of \mathbf{m} . Let \mathbf{m}'_0 be an ordering of \mathbf{m}' and denote by (r_1, \dots, r_{k-1}) the ordered vertices of \mathbf{m}'_0 . \mathbf{m}_0 then denotes the ordering (r_1, \dots, r_{k-1}, s) of \mathbf{m} .

Consider any ordered set $U = (u_1, \dots, u_{k-1})$ of $k - 1$ vertices in $V(G)$ and define the random variable $N_U(\mathbf{m})$ as the number of copies of \mathbf{m} in G whose restriction on U is isomorphic to \mathbf{m}'_0 as an ordered motif (see Figure 2 for an example). Our aim is to bound the tail of the distribution of $N_U(\mathbf{m})$.

Let us denote by $Y_U(\mathbf{m}'_0)$ the indicator of an occurrence of \mathbf{m}'_0 on U as an ordered motif, that is

$$Y_U(\mathbf{m}'_0) = \mathbb{I}_{G[U] \sim \mathbf{m}'_0}.$$

For each vertex $v \notin U$, let $\text{ext}_U^v(\mathbf{m}'_0, \mathbf{m}_0)$ be the indicator of the fact that the extension from $G[U]$ to $G[U \cup \{v\}]$ is isomorphic to the extension of \mathbf{m}'_0 to \mathbf{m}_0 with respect to the order of the vertices. Denoting by e_{ab} the indicator function for the edge from vertex a to vertex b in \mathbf{m}_0 ,

$$\text{ext}_U^v(\mathbf{m}'_0, \mathbf{m}_0) = 1 \Leftrightarrow X_{vv} = e_{ss} \text{ and } \forall i, X_{u_i v} = e_{r_i s} \text{ and } X_{vu_i} = e_{sr_i}$$

Note that $\text{ext}_U^v(\mathbf{m}'_0, \mathbf{m}_0)$ only depends on the edges between v and U and is therefore independent of $Y_U(\mathbf{m}'_0)$. Moreover, if $G[U]$ corresponds to an occurrence of \mathbf{m}'_0 and that $\text{ext}_U^v(\mathbf{m}'_0, \mathbf{m}_0) = 1$, $G[U \cup \{v\}]$ corresponds to an occurrence of \mathbf{m}_0 . In that case we say that the occurrence of \mathbf{m}_0 on $U \cup \{v\}$ is a *valid extension* of the occurrence of \mathbf{m}'_0 on U .

Moreover, as $N_U(\mathbf{m})$ is null when there is no occurrence of \mathbf{m}'_0 on U and as, if \mathbf{m}'_0 occurs on U , $N_U(\mathbf{m})$ counts the number of its valid extensions, we have

$$N_U(\mathbf{m}) = Y_U(\mathbf{m}'_0) \sum_{v \notin U} \text{ext}_U^v(\mathbf{m}'_0, \mathbf{m}_0) \quad (2)$$

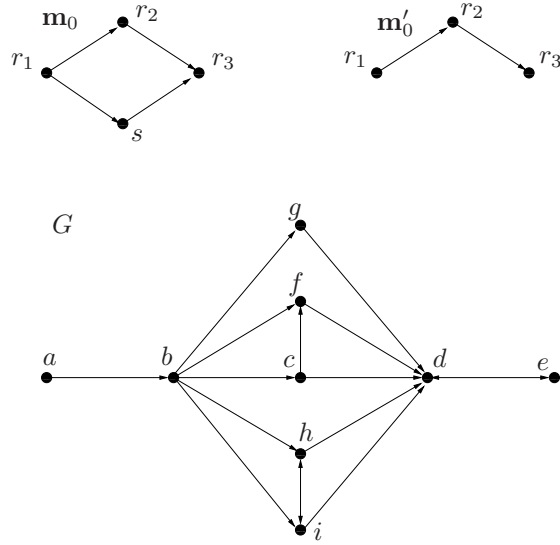


Figure 2: For $U = (b, c, d)$, $Y_U(\mathbf{m}'_0) = 1$ and $N_U(\mathbf{m}) = 3$. Indeed, one obtains valid extensions of \mathbf{m}'_0 to \mathbf{m}_0 by adding to U the vertices g , h or i . Adding the vertex f does not give rise to a valid extension because of the presence of an edge from c to f .

Bounding the tail of the distribution of $N_U(\mathbf{m})$ can be done by using the fact that either $Y_U(\mathbf{m}'_0) = 0$ and $N_U(\mathbf{m})$ is null or $Y_U(\mathbf{m}'_0) = 1$ and $N_U(\mathbf{m})$ is a sum on independent Bernoulli trials. In the latter case, changing one of the Bernoulli trials affects $N_U(\mathbf{m})$ by at most 1. Numerous results show that random variables showing such characteristics are highly concentrated around their mean value. One of them is the following theorem [16]:

Theorem 1. *Let the random variables X_1, \dots, X_n be independent, with $0 \leq X_k \leq 1$ for each k , and let $S_n = \sum X_k$. Then, for every $t > 0$,*

$$\mathbb{P}\left(\frac{S_n - \mathbb{E}S_n}{\mathbb{E}S_n} > t\right) \leq e^{-((1+t)\ln(1+t)-t)\mathbb{E}S_n}$$

We will use this theorem to bound the p-value of seeing, at any position U , an occurrence of \mathbf{m}'_0 having a number of valid extensions to \mathbf{m}_0 which is large with respect to the expected number of valid extensions.

In that purpose, let us denote by $Ext_U(\mathbf{m}'_0, \mathbf{m}_0)$ the mean number of valid extensions of a putative occurrence of \mathbf{m}'_0 on U , that is

$$Ext_U(\mathbf{m}'_0, \mathbf{m}_0) = \mathbb{E} \sum_{v \notin U} ext_U^v(\mathbf{m}'_0, \mathbf{m}_0)$$

For the following, in order to simplify the notations, we will write Ext_U instead of $Ext_U(\mathbf{m}'_0, \mathbf{m}_0)$ when there is no ambiguity.

The main result of the paper is now the following theorem, which shows that the probability of finding a position U such that $N_U(\mathbf{m})$ is much larger than Ext_U decreases exponentially:

Theorem 2. *Let h be the function defined on $[0, +\infty[\times]0, +\infty[$ by*

$$h(X, Y) = \begin{cases} 0 & \text{if } X \leq Y \\ X \ln\left(\frac{X}{eY}\right) + Y & \text{otherwise} \end{cases}$$

Then, for every $t > 0$,

$$\mathbb{P}\left(\max_U(h(N_U(\mathbf{m}), Ext_U)) > t\right) \leq aut(\mathbf{m}') \mathbb{E}N(\mathbf{m}') e^{-t}$$

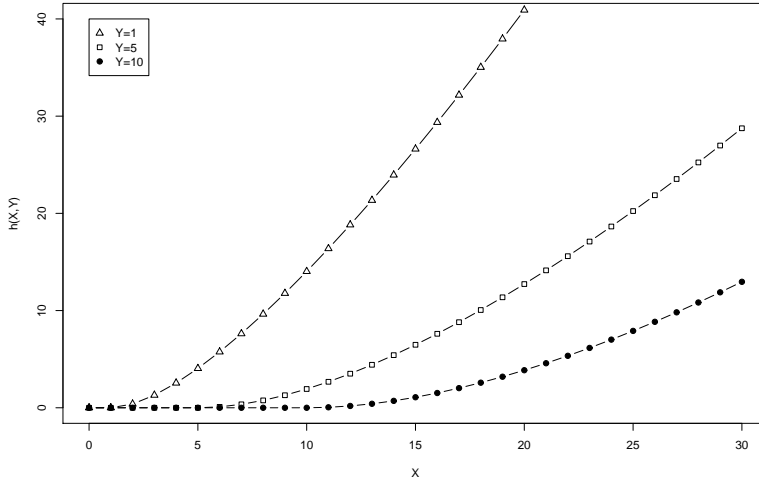


Figure 3: Curves of the function h for fixed values of Y

Note that for a fixed value of Y , $h_Y : X \rightarrow h(X, Y)$ is an increasing function, growing asymptotically as $X \ln(X)$, as illustrated in Figure 3.

Therefore, the inequality of Theorem 2 bounds the probability of seeing any large $N_U(\mathbf{m})$, as

$$\max_U(h(N_U(\mathbf{m}), Ext_U)) > t \Leftrightarrow \exists U / h(N_U(\mathbf{m}), Ext_U) > t$$

To illustrate this, consider the particular case where the probabilistic event considered is $\{\exists U / N_U(\mathbf{m}) \geq e^2 Ext_U + t\}$, for some positive t . It is straightforward to see that

$$\begin{aligned} N_U(\mathbf{m}) \geq e^2 Ext_U + t &\Rightarrow \ln\left(\frac{N_U(\mathbf{m})}{e Ext_U}\right) > 1 \\ &\Rightarrow h(N_U(\mathbf{m}), Ext_U) > N_U(\mathbf{m}) \\ &\Rightarrow h(N_U(\mathbf{m}), Ext_U) > t. \end{aligned}$$

The following corollary of Theorem 2 follows:

Corollary 1. *For every $t > 0$,*

$$\mathbb{P}(\exists U/N_U(\mathbf{m}) \geq e^2 Ext_U + t) \leq aut(\mathbf{m}') \mathbb{E}N(\mathbf{m}') e^{-t}$$

Proof of Theorem 2:

Let us first introduce a new function g and a technical result which will be useful later on:

Lemma 1. *Let g be the function defined by*

$$g: \begin{array}{ll} [0, +\infty[& \rightarrow [0, +\infty[\\ y & \rightarrow (1+y) \ln(1+y) - y \end{array}$$

i) g is increasing

ii) g is one-to-one

iii) For every $X \geq 0, Y > 0$,

$$g\left(\max\left(\frac{X-Y}{Y}, 0\right)\right)Y = h(X, Y)$$

Proof: i) and ii) are straightforward. To prove iii), let us consider $X \geq 0$ and $Y > 0$. Suppose that $X \leq Y$. Then

$$g\left(\max\left(\frac{X-Y}{Y}, 0\right)\right)Y = g(0)Y = 0 = h(X, Y).$$

On the other hand, if $X > Y$,

$$\begin{aligned} g\left(\max\left(\frac{X-Y}{Y}, 0\right)\right)Y &= g\left(\frac{X-Y}{Y}\right)Y \\ &= \left(\left(1 + \frac{X-Y}{Y}\right) \ln\left(1 + \frac{X-Y}{Y}\right) - \frac{X-Y}{Y}\right) \cdot Y \\ &= X \ln\left(\frac{X}{Y}\right) - X + Y \\ &= h(X, Y). \end{aligned}$$

■

To prove Theorem 2, which is a result giving an exceptionality measure on the whole graph, we start by showing a local exceptionality result. To do so, we fix a position U and show the following result:

Lemma 2. *For every $t > 0$,*

$$\mathbb{P}(h(N_U(\mathbf{m}), Ext_U) > t) \leq \mathbb{P}(Y_U(\mathbf{m}'_0) = 1) e^{-t}.$$

Proof:

Let us first decompose the event of interest by looking for an occurrence of \mathbf{m}' on U :

$$\begin{aligned} \mathbb{P}(h(N_U(\mathbf{m}), Ext_U) > t) &= \mathbb{P}(h(N_U(\mathbf{m}), Ext_U) > t | Y_U(\mathbf{m}'_0) = 1) \mathbb{P}(Y_U(\mathbf{m}'_0) = 1) \\ &\quad + \mathbb{P}(h(N_U(\mathbf{m}), Ext_U) > t | Y_U(\mathbf{m}'_0) = 0) \mathbb{P}(Y_U(\mathbf{m}'_0) = 0) \end{aligned}$$

If $Y_U(\mathbf{m}'_0) = 0$, then $N_U(\mathbf{m}) = 0$ and thus $h(N_U(\mathbf{m}), Ext_U) = 0$. Therefore,

$$\mathbb{P}(h(N_U(\mathbf{m}), Ext_U) > t) = \mathbb{P}(h(N_U(\mathbf{m}), Ext_U) > t | Y_U(\mathbf{m}'_0) = 1) \mathbb{P}(Y_U(\mathbf{m}'_0) = 1). \quad (3)$$

Defining $y = g^{-1}\left(\frac{t}{Ext_U}\right)$, we have:

$$\begin{aligned} &\mathbb{P}(h(N_U(\mathbf{m}), Ext_U) > t | Y_U(\mathbf{m}'_0) = 1) \\ &= \mathbb{P}\left(g\left(\max\left(\frac{N_U(\mathbf{m}) - Ext_U}{Ext_U}, 0\right)\right) Ext_U > t | Y_U(\mathbf{m}'_0) = 1\right) \text{ by Lemma 1 iii)} \\ &= \mathbb{P}\left(g\left(\max\left(\frac{N_U(\mathbf{m}) - Ext_U}{Ext_U}, 0\right)\right) > g(y) | Y_U(\mathbf{m}'_0) = 1\right) \\ &= \mathbb{P}\left(\max\left(\frac{N_U(\mathbf{m}) - Ext_U}{Ext_U}, 0\right) > y | Y_U(\mathbf{m}'_0) = 1\right) \text{ by Lemma 1 i)} \\ &= \mathbb{P}\left(\frac{N_U(\mathbf{m}) - Ext_U}{Ext_U} > y | Y_U(\mathbf{m}'_0) = 1\right) \text{ as } y > 0 \\ &= \mathbb{P}\left(\frac{\sum_{v \notin U} ext_U^v(\mathbf{m}'_0, \mathbf{m}_0) - Ext_U}{Ext_U} > y | Y_U(\mathbf{m}'_0) = 1\right) \text{ by using (2)} \\ &= \mathbb{P}\left(\frac{\sum_{v \notin U} ext_U^v(\mathbf{m}'_0, \mathbf{m}_0) - Ext_U}{Ext_U} > y\right) \end{aligned} \quad (4)$$

where the last equation is due to the fact that each $ext_U^v(\mathbf{m}'_0, \mathbf{m}_0)$ is independant from $Y_U(\mathbf{m}'_0)$.

Theorem 1 can be now applied to the random variables $(ext_U^v(\mathbf{m}'_0, \mathbf{m}_0))_{v \notin U}$ and therefore implies that

$$\mathbb{P}\left(\frac{\sum_{v \notin U} ext_U^v(\mathbf{m}'_0, \mathbf{m}_0) - Ext_U}{Ext_U} > y\right) \leq e^{-g(y)Ext_U}$$

that is, as $g(y)Ext_U = t$,

$$\mathbb{P}(h(N_U(\mathbf{m}), Ext_U) > t | Y_U(\mathbf{m}'_0) = 1) \leq e^{-t} \quad (5)$$

Using Inequality (5) in Equation (3) yields Lemma 2. ■

To prove the main theorem, one has to note that

$$\{\max_U(h(N_U(\mathbf{m}), Ext_U) > t)\} = \bigcup_U \{h(N_U(\mathbf{m}), Ext_U) > t\}$$

Therefore,

$$\begin{aligned} \mathbb{P}(\max_U(h(N_U(\mathbf{m}), Ext_U) > t) &\leq \sum_U \mathbb{P}(h(N_U(\mathbf{m}), Ext_U) > t) \\ &\leq \sum_U \mathbb{P}(Y_U(\mathbf{m}'_0) = 1)e^{-t} \\ &\leq aut(\mathbf{m}')\mathbb{E}N(\mathbf{m}')e^{-t} \text{ by using (1)} \end{aligned}$$

■

5 Application to the gene regulation network of Yeast

The results of Section 4 allow us to decide if a motif \mathbf{m} is considered as being over-represented in a real graph with respect to at least one of its submotifs. To do so, we use the following steps:

- Choose a random graph model and estimate its parameters on the real graph.
- Choose a deletion class in \mathbf{m} and consider the obtained submotif \mathbf{m}' .
- List all the occurrences of \mathbf{m} and thus all the occurrences of \mathbf{m}' having at least one valid extension. Then, for every occurrence \mathbf{m}'_0 in that list, count the number of its valid extensions $N_U(\mathbf{m})$ and compute the mean value $\mathbb{E}(N_U(\mathbf{m}))$, where U is the vertex set of \mathbf{m}'_0 .
- Compute the bound of the p-value using Theorem 2. Having listed all positions U of interest and the associated values of $N_U(m)$ allows the user to have access to the positions where the local concentration takes place.

The first step needs to be done only once when dealing with a whole list of motifs whereas the three last ones have to be done for every deletion class of every motif of interest.

This method is applied to the gene regulation network of Yeast, available at Uri Alon's Lab website [11]. That network has 688 vertices and 1078 edges and a directed edge between genes g_1 and g_2 denotes a regulation from gene g_1 on the expression of gene g_2 . We don't take the type of regulation into account here, that is if it is an activation or an inhibition.

The random graph model we consider is the Mixture Model with fixed classes. In that model, the n vertices are spread into Q classes.

We consider a matrix $\Pi = (\pi_{qr})_{1 \leq q, r \leq Q}$ which gives the connection probabilities between classes and for each pair (i, j) of vertices, we define $c_{ij} = \pi_{qr}$, q and r being the respective classes of i and j . In other words, the connectivity probability between two vertices only depends on their mutual classes.

To estimate the partition of the graph and the corresponding matrix Π , we use the algorithm developed by Latouche and al. [14], and assign each vertex to its most probable class.

Table 1 shows the unique motif of size three detected. The column *p-value bound* contains the upper bound of the real p-value given by Theorem 2 for $t = \max_U(h(N_U(\mathbf{m}), Ext_U))$, whereas the *Agglomeration* coefficient is the maximal observed value of $N_U(\mathbf{m})$.

That motif, called the feed-forward loop, is known [2] to play a role in regulation processes by inducing a delay in the regulation of Z by X : X has first to activate Y and then X and Y together regulate Z . Note that X , Y and Z are not in the same deletion class, as mentioned in Section 2, and there are therefore three different submotifs to take into account, even if each of them is a single edge.

Our analysis finds the feed-forward loop to be over-represented and shows that there are genes X using the same gene Y to regulate a high number of genes Z (up to 15). On the contrary, there is no gene X using a high number of intermediates Y to regulate a given gene Z .

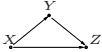
Motif	Deletion class	p-value bound	Agglomeration
	X	$5.15e - 3$	3
	Y	3.33	2
	Z	$1.12e - 11$	15

Table 1: p-values for the three possible sub-motifs of the feed-forward loop

Table 2 shows all the motifs of size four which are found to be over-represented with respect to at least one of their submotifs.

The motif of size four with the lowest p-value is the bi-fan, that is the first motif shown in table 2. That motif consists in two regulators having an impact on two common genes. It was first shown to be over-represented in that network by Milo and al [17] and appears first in all motif detection algorithms.

Nevertheless, our approach gives a supplementary information, that is that it is highly over-represented with respect to the sub-motif obtained by suppressing one of the regulated genes. In other words, there exist in Yeast co-regulators which co-regulate a high number of genes simultaneously. The agglomeration coefficient shows that they may act on up to 37 common genes. On the other hand, the bi-fan is *not* over-represented with respect to the sub-motif obtained by suppressing one of the regulators, that is there exist no couple of genes that are influenced by a high number of common regulators. In fact, running the

algorithm in that case leads to a p-value of .18 and an agglomeration coefficient of 4. That assymetry between couples of regulators and couples of regulated genes is well known by biologists but is found again here only by statistical means.

Note also that among the six detected motifs, the third and the two last ones are in fact by-products of the over-representation of the feed-forward loop: they are not overrepresented with respect to their feed-forward loop submotif, that is the one obtained by deleting T .

From a computational point of view, we obtain a significative improvement in terms of running time. Indeed, available methods are of two kinds: either they use a reasonable number of simulations and use a Z-score and are quite rapid but may lead to false positives, or they make use of a huge number of simulated graphs to obtain an empirical p-value but are very time-consuming. The part of our method which takes the most time is in fact the preprocessing by the algorithm of Latouche et al. [14] to estimate the parameters of the mixture model. The concentration part is very rapid as it only needs to compute expectations, which are easy to calculate in mixture models. Running our algorithm and the empirical p-value option of the MFinder tool [17] for the graphs of size four in the Yeast network divides the running time by a factor 100 (less than 10 minutes compared to more than 15 hours on a dual core).

6 Conclusion

We develop a new method to detect network motifs by looking for local over-representations. To do so, we show that, given an occurrence of a submotif, the number of motifs extending it is highly concentrated around its mean value. That approach allows to consider the over-representation with respect to a submotif and to avoid time-consuming simulations.

Acknowledgements

The author would like to thank C. Matias for her helpful comments.

References

- [1] E. Airoldi, D. Blei, S. Fienberg, and E. Xing. Mixed membership stochastic blockmodels. *Journal of Machine Learning Research*, 9:1981–2014, 2008.
- [2] U. Alon and al. Network motifs: theory and experimental approaches. *Nature Reviews Genetics*, 8:450–461, 2007.
- [3] A.-L. Barabási and R. Albert. Emergence of scaling in random networks. *Science*, 286:509–512, 1999.

-
- [4] E. Birmelé. A scale-free graph model based on bipartite graphs. *Discrete Applied Mathematics*, to appear.
 - [5] B. Bollobás, S. Janson, and O. Riordan. The phase transition in inhomogeneous random graphs. *Random structures and algorithms*, 31:3–122, 2007.
 - [6] S. Boucheron and al. Concentration inequalities using the entropy method. *Annals of Probability*, 31:1583–1614, 2003.
 - [7] J.-J. Daudin and al. Mixture model for random graphs. *Statistics and computing*, In press, 2008.
 - [8] R. Dobrin and al. Aggregation of topological motifs in *escherischia coli* transcriptional regulatory network. *BMC Bioinformatics*, 5:10, 2004.
 - [9] P. Erdős and A. Rényi. On random graphs i. *Publ. Math. Debrecen*, 6:290–297, 1959.
 - [10] J. Hofman and C. Wiggins. A bayesian approach to network modularity. *Physical Review Letters*, 100, 2008.
 - [11] <http://www.weizmann.ac.il/mcb/UriAlon/>.
 - [12] N. Kashtan and al. Efficient sampling algorithm for estimating subgraph concentrations and detecting network motifs. *Bioinformatics*, 20-11:1746, 2004.
 - [13] J.H. Kim and V.H. Vu. Divide and conquer martingales and the number of triangles in a random graph. *Random structures and algorithms*, 24:166–174, 2004.
 - [14] P. Latouche, E. Birmelé, and C. Ambroise. Bayesian methods for graph clustering. *preprint SSB*, 2008.
 - [15] C. Matias, S. Schbath, E. Birmelé, J.J. Daudin, and S. Robin. Network motifs: mean and variance for the count. *Revstat*, 4(1):31–51, 2006.
 - [16] C. McDiarmid. Concentration. In J. Ramirez-Alfonsin B. Reed (Eds.) M. Habib, C. McDiarmid, editor, *Probabilistic Methods for Algorithmic Discrete Mathematics*, pages 195–248. Springer, 1998.
 - [17] R. Milo and al. Network motifs: Simple building blocks of complex networks. *Science*, 298:824–827, 2002.
 - [18] M. Molloy and B. Reed. A critical point for random graphs with a given degree sequence. *Random Structures and Algorithms*, 6:161–179, 1995.
 - [19] F. Picard and al. Assessing the exceptionality of network motifs. *Journal of Computational Biology*, 15, 2008.
 - [20] S. Wernicke and F. Rasche. Fanmod: a tool for fast network motif detection. *Bioinformatics*, 22(9):1152–1153, 2006.

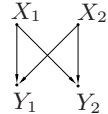
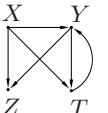
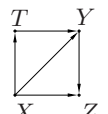
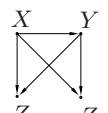
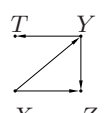
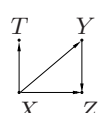
Motif	Deletion class	p-value bound	Agglomeration
	$\{Y_1, Y_2\}$	$1.60e - 25$	37
	Z	$3.33e - 16$	15
	Z	$2.22e - 13$	15
	$\{Z_1, Z_2\}$	$4.77e - 12$	14
	Z	$8.35e - 12$	15
	Z	$2.12e - 10$	15

Table 2: Over-represented motifs of size four