# Genome-Wide Detection of *sRNA* Targets with *rNAV*

Jonathan Dubois[*]
LABRI UMR CNRS 5800,
Université Bordeaux 1,
France

Amine Ghozlane[†]
DSIMB, INSERM
UMR-S 665, Université
Paris 7, INTS, France

Patricia Thébault[‡]
CGFB - CBiB and LABRI
UMR CNRS 5800,
Université Bordeaux 2,
France

Isabelle Dutour[§]
LABRI UMR CNRS 5800,
Université Bordeaux 1,
France

Romain Bourqui[¶]
LABRI UMR CNRS 5800,
Université Bordeaux 1,
France

**ABSTRACT**

The central dogma in molecular biology postulated that 'DNA makes RNA makes protein', however this dogma has been recently extended to integrate new biological activities involving small bacterial noncoding RNAs, called *sRNA*s. Accordingly, increasing attention has been given to these molecules over the last decade and related experimental works have shown a wide range of functional activities for these molecules. In this paper, we present *rNAV* (for *rna NAVigator*), a new tool for the visual exploration and analysis of bacterial sRNA-mediated regulatory networks. *rNAV* has been designed to help bioinformaticians and biologists to identify, from lists of thousands of predictions, pertinent and reasonable sRNA target candidates for carrying out experimental validations. We propose a list of dedicated algorithms and interaction tools that facilitate the exploration of such networks. These algorithms can be gathered into pipelines which can then be saved and reused over several sessions. To support exploration awareness, *rNAV* also provides an exploration tree view that allows to navigate through the steps of the analysis but also to select the sub-networks to visualize and compare. These comparisons are facilitated by the integration of multiple and fully linked views. We demonstrate the usefulness of our approach by a case study on *Escherichia coli* bacteria performed by domain experts.

**Index Terms:** I.3.6 [Computer Graphics]: Methodology and Techniques—Interaction techniques; J.3 [Computer Applications]: Life and Medical Sciences—Biology and genetics

## 1 INTRODUCTION

The central dogma in molecular biology postulated that 'DNA makes RNA makes protein'. More precisely, a first process called transcription creates an *mRNA* (*messenger* RNA) copy from one specific region of the DNA molecule, called gene. Then, the mRNA is used to synthesize a protein through a process called translation. This dogma has been recently extended to integrate new biological activities [34] involving, for instance, the small noncoding RNAs (*sRNA*s). Accordingly, an increasing attention has been given to these molecules over the last decade and related experimental works have revealed a wide range of new functional activities for these molecules [32, 39] in bacteria. Among them, RNAs are involved in regulating biological processes like transcription, translation, mRNA stability and DNA maintenance or silencing [31]. Of particular interest, these molecules are known to rapidly modulate the cell behavior and fitness according to environmental stimuli. The single-stranded molecule structure of a sRNA allows some regions of its sequence to form base-pairing with another *RNA* molecule [40, 44]. That folding between one sRNA and its mRNA targets modify the structure and the stability of mRNAs to positively or negatively regulate their translation into protein or even their stability.

With the extraordinary increase in sequencing capacity through new sequencing technologies (NGS), in combination with specialized mRNA enrichment and tiling array techniques [12, 39], many prokaryotic transcriptomes (*e.g. Escherichia coli, Bacillus subtilis*) have been explored and have revealed the existence of a plethora of small regulatory RNAs. The identification/inference of these sRNA functions, investigating their mRNA targets, implies carrying out time-consuming and expensive experimental approaches [43]. This is especially true for bacteria that are fastidious to grow and have a highly divergent genome composition. Hence, the bioinformatics community focuses on the design of fast and inexpensive *in silico* approaches to prioritize gene candidates before designing an experimental protocol. However, the development of these approaches is still challenging as classical bioinformatics approaches are often inefficient in term of sensitivity and the number of false predictions can be significant. To address this issue and reduce the number of sRNA-mRNA interactions to inspect, it is crucial to exploit biological knowledge through appropriate filters.

In this paper, we present *rNAV*, a visualization tool designed to help bioinformaticians and biologists to identify, from lists of thousands of predictions, pertinent and reasonable sRNAs and their target candidates for carrying out experimental validations. We introduce an integrative approach that fuses sRNA-mRNA relationships with multi-purposed annotation databases. In other words, we propose to visually exploit the mRNA-target information to filter out the false-positive predictions using:

- their functional activity when known,
- the pairing region involved within the mRNA or the sRNA,
- the neighborhood information in the entire network.

Our combined approach, exploiting the information of interacting duplexes, eases the task of characterizing the regulatory role of newly discovered sRNAs. While some research has been made for the visual mining of biological networks, like signaling network [5], protein-protein network [18] or metabolic network [33] (for a review see [27]), there has been, to the best of our knowledge, no previously published work on providing visual support for the prediction of sRNA-mRNA interactions at a genome scale. Integration of biological databases is currently one the main challenges in the data mining community, and many works have focused on enrichment analyses for driving the integration of multipurpose omics or

[*]e-mail: jonathan.dubois@labri.fr
[†]e-mail: amine.ghozlane@inserm.fr
[‡]e-mail: patricia.thebault@labri.fr
[§]e-mail: isabelle.dutour@labri.fr
[¶]e-mail: romain.bourqui@labri.fr

IEEE Symposium on Biological Data Visualization 2013
October 13 - 14, Atlanta, Georgia, USA

Table 1: Questions/requirements for visual mRNA-targets identification with corresponding data/task abstractions and visual encodings, algorithms and interaction tools.

| | Domain problem/question | Data/Task abstraction | Visual Encoding/Algorithm/Interaction |
|---|---|---|---|
| P1 | Analyse the entire regulatory network | Provide a visualization of the network | Nodelink diagram representation of the network |
| P2 | Detection of regulatory (SIM or DOR) motifs | Visualize node's neighborhood ; Detect common neighborhood of nodes of the network | Support the visualization of neighborhood with our dedicated Bring & Go interaction tool ; Provide shortest paths algorithm and support extraction of sub-networks of interest |
| P3 | Exploitation of experts biological knowledge (*e.g.* mRNAs name, database, annotations) | Provide elementary filters based on attributes | Provide filter/selection algorithm to filter out elements according to their name, annotations and/or database including them |
| P4 | Identification of evolutionary constrained regions, *i.e.* regions with a high number of interactions | Identify nodes based on their adjacent edges information | Provide filter/selection algorithm to identify RNAs according to their interacting regions ; For each edge, show as label the interacting regions of its extremities ; Support selection of a RNA's neighborhood according to the region of interest ; Support the visualization for a given RNA of the number of interactions each base is involved in ; Provide a clustering algorithm to group for each node its adjacent edges and visually render it as a glyph |
| P5 | Reproduce the analysis while modifying some parameters | Keep track of previous analysis pipeline | Save/Reuse analysis pipelines |
| P6 | Observe/Study/Compare different filters and pipelines | Apply filters/pipelines several times on the same -sub-network ; Detect common elements in the resulting -sub-networks | Provide multiple linked views |
| P7 | Very long analysis | Support multiple sessions analysis & Keep track of previous analysis | Import/update/save an exploration tree view to give access to previous states of the analysis within/across multiple sessions |

annotated data (for a review see [15]). However few of them integrate visualization approaches (for examples see [29]), and if so, they are dedicated to specific biological networks with a single database (*e.g.* [3, 14, 24, 7, 22]). Keeping in mind that one of the advantages of visualization is based on filtering processes to iteratively reduce the scope of the analysis, the integration of enriched methods to incorporate as much biological knowledge as possible is of great importance to drive more realistic analyses.

The remainder of this paper is structured as follows. In section 2, we first introduce the network construction and then present the underlying analytic process and requirements needed by our visualization software to fulfill. We next describe *rNAV*, a new visualization tool integrating all these requirements in section 3. We then explain how *rNAV* helped our bioinformatician collaborators to identify pertinent sRNA-mRNA interactions in the *Escherichia coli* bacteria. Finally, we draw a conclusion and give directions for future work in section 5.

## 2 Genome-wide detection of sRNA targets: data generation and requirements

In this section, we first describe the prediction pipeline for the data generation. We then describe the main biological questions and related tasks, and we provide a list of requirements our tool try to satisfy.

### 2.1 An Integrative Prediction Pipeline to Build Regulatory RNA Networks

The main objective of the prediction pipeline is to provide sRNA-mRNA interaction networks that will guide biologists in prioritizing gene and target candidates. As a first step, putative sRNA-mRNA interactions are predicted and then additional functional information are assigned to mRNAs molecules. To carry out this two pipeline steps, we combined two well-known tools, respectively IntaRNA [8] and DAVID [17, 16]. While IntaRNA supports the detection of putative sRNA-mRNA interactions, DAVID [17, 16] performs gene annotation enrichments using statistical approaches.

For a given organism, we first use as input sRNAs sequences (derived from the results of RNA-Seq experiments), and the complete list of annotated mRNAs of the corresponding genome.

For computing the first level of predicted data, we chose IntaRNA [8] as it offers a good trade-off between specificity and sensitivity. This software integrates two algorithms for the detection of putative interactions. While the first method has a $O(n^2m^2)$ time complexity (where $n$ and $m$ are the lengths of the two interacting RNAs) and is not suitable for genome-wide predictions, the heuristic version has a quadratic time complexity. To further improve computation times, we developed a multi-core implementation of this algorithm. At the end of this step, we have for each input sRNA a collection of putative interacting mRNAs as well as the positions of the sequences involved in these interactions.

The second stage aims to propose a functional annotation to each sRNA on the basis of their putative mRNA targets. To do so, we used DAVID [17, 16], a single gene-term enrichment analysis software. This approach takes as input the collections of mRNAs corresponding to each sRNA and calculates mRNAs sub-groups based on the presence of a statistically significant over-represented annotation. DAVID also offers the advantage to exploit several biological knowledge databases to provide different annotation concepts:

- GO (Biological Process, Molecular Function and Cellular Component) http://www.geneontology.org
- COG/KOG Ontology http://www.ncbi.nlm.nih.gov/COG/new
- SMART Domains http://smart.embl-heidelberg.de
- InterPro Domains http://www.ebi.ac.uk/interpro

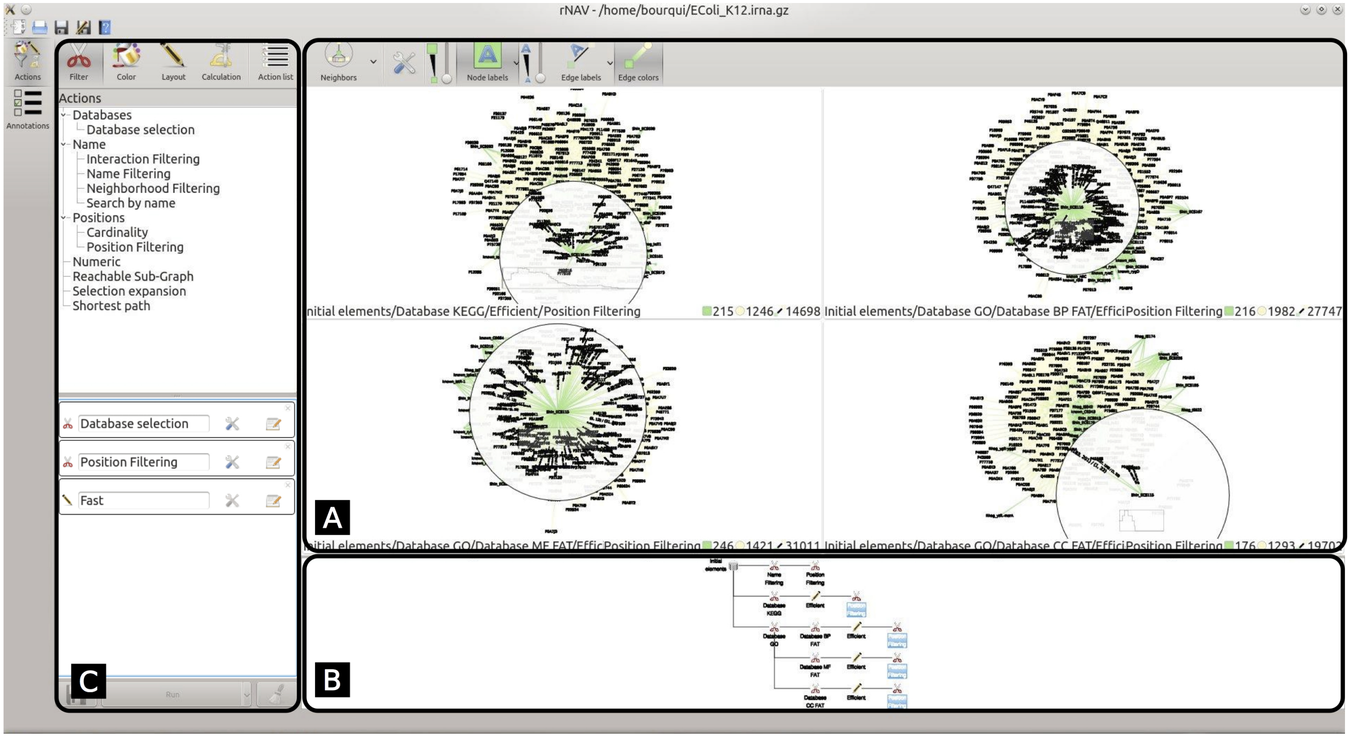



Figure 1: Screenshot of our software after few analysis steps. Panel (A) contains the visualization widget and interaction tools, here four sub-networks are represented and our dedicated *Bring & Go* is used on an element. The neighborhood of that element is highlighted in all views. Panel (B) contains the exploration tree view. In that tree, the root node corresponds to the original data and each time an algorithm is applied on a node (*i.e.* a -sub-network), we create a son in the tree corresponding to the output of the algorithm. The exploration tree view is used to select the -sub-networks to visualize in Panel (A). In Panel (C), one can see the list of available algorithms (top) categorized into *Filter*, *Color*, *Layout* and *Calculation* and the algorithms pipeline editor (bottom). Saved algorithm pipelines can be retrieved from the *Action list* tab. Clicking on the *Run* button will run all the algorithms of the pipeline editor on each visualized/selected -sub-network (from the exploration tree view).

- KEGG Pathways http://www.genome.jp/kegg
- UniProt Sequence Features http://www.pir.uniprot.org
- PIR SuperFamily Names http://pir.georgetown.edu/iproclass

For instance, KEGG annotations [21] are helpful to understand the metabolic function of proteins that encodes for enzymes. An other example is the Gene Ontology database [1] where biological information associated to a gene is defined by a set of functional terms related by relationships within a directed acyclic graph. For a group of mRNAs related to a given sRNA, the over-represented biological annotations might be considered as a putative sRNA's annotation. These annotations are thus not related to the sRNA but rather to the combination of sRNA-mRNA interactions with an enrichment score for significance of these annotations.

The data generated by the combination of IntaRNA and DAVID tools are naturally modelized by a bipartite graph where the two sets of nodes are respectively the sRNAs and the mRNAs. Two RNAs are linked by an edge if and only if they were predicted as interacting molecules. Edges of that graph, linking sRNAs and mRNAs, are labeled with the annotations retrieved from various databases (*e.g.* GO, KEGG or UniProt). Another information of utmost importance is given by the base-pairing region of the sequences involved in each putative sRNA-mRNA interaction.

### 2.2 Domain Problems and Requirements

Focusing on a very local level of the sRNA-mediated regulatory network, Beisel and Storz [4] have suggested a classification describing regulatory motifs according to their topology but also their biological impact for the cell. The *SIM* motif (Single-Input Module), required by a single external perturbation, corresponds to one sRNA regulating several mRNAs. Conversely, the *DOR* motif (Dense Overlapping Regulon) represents a coordinated response of the cell to several stress conditions. It therefore involves the regulation of mRNAs towards several sRNAs, and vice versa. Moreover Modi *et al* [25] have modeled the sRNA-mediated regulatory network of *Escherichia coli* . They provided in addition to their model, predictive metabolic information and identified sRNA-centred clusters where interacting mRNAs showed an enriched annotation with a similar function. Co-evolution information might also be exploited to predict functions of sRNAs. As evolution of these regions may have been constrained, biologists believe them to have a high number of interactions.

Based on discussions with our collaborators, we identified 7 main domain problems and questions, and formulated the corresponding visualization requirements. Table 1 provides the domain questions, task abstractions and the corresponding features our tool integrates to fulfill these requirements.

One of the aims is to generalize the methodology of [4, 25] to entire networks, our tool therefore needs to support the visualization of the whole network (P1 in Table 1). Then, to perform similar analyses, bioinformaticians have to first focus on few RNAs to investigate, and second to combine topology information (P2 in Table 1) with biological knowledge (P3 in Table 1). Dedicated selection/filtration algorithms have to be integrated to extract elements of interest based on topology (*e.g.* the shortest paths between two RNAs) but also on biological knowledge to initiate the exploration and/or to validate the resulting motifs. Concerning co-evolution information (P4 in Table 1), the detection of such regions can be achieved by identifying regions with a high number of interactions. It requires to visualize the interacting regions for single edge as well as the number of interactions for each base of a RNA. To further facilitate the identification of such regions, we also propose a clustering algorithm which groups for each RNA its putative interactions according their interacting regions. Problems P5 and P6 are due to the reproducibility and the exploratory aspect of such analyses. In order to reproduce an analysis pipeline while modifying parameters, it is important to support their editing and the reuse of these pipelines. Modifying the parameters may produce different results that need to be compared. At last, carrying out such a long analytic process at once can be tedious (P7 in Table 1) and may require multiple analysis sessions. To remind the user his previous analysis steps, it is thus necessary to keep track of them.

## 3 rNAV SOFTWARE

In this section, we present *rNAV*, a visualization software that is dedicated to the visual exploration of the sRNA-mediated regulatory network generated by a combination of experimental/predictive data. *rNAV* has been developed in C++ with the Tulip framework [2]. Tulip provides some of the algorithms and widgets integrated in *rNAV* (*e.g.* betweenness centrality [10] , Jaccard's index [19], GEM [11], $FM^3$ [13], MCL [41] or the nodelink diagram widget). While these algorithms/widget just needed to be set correctly, other were especially developed for *rNAV* (*e.g.* a dedicated *Bring & Go* [26] or the exploration tree widget). In other words, *rNAV* takes full advantage of the Tulip plugins management system and its list of features can therefore be extended if necessary.

### 3.1 Network Visualization

As mentioned above, the input of *rNAV* is a list of sRNAs and mRNAs as well as putative interactions linking them. In that data, we can distinguish two sets of elements: the set of all mRNAs and the set of all sRNAs. An intuitive data modeling is therefore a bipartite graph modeling where the two sets of elements correspond to the two types of RNAs. In addition, our predictive pipeline provides enriched annotations for the putative interactions. In Panel (A) of Figure 1, one can see nodelink diagram views on the *Escherichia coli K12* network. In each view, mRNAs are represented by green circles while mRNAs are represented by yellow circles. Edges labels (respectively nodes labels) allow to show the enriched annotations (respectively RNA names). However labels can be used to display any other input information, like the p-values associated to the putative interactions or the interacting regions. Furthermore, nodes sizes and nodes labels sizes can be interactively modified to fit them with the current layout of the network (see Section 3.2).

### 3.2 Algorithms and Analysis Pipelines

To answer the domain questions or problems (P2, P3 and P4 in Table 1), *rNAV* provides a list of algorithms. To simplify the access to the different algorithms, we classified them into four categories: *Filter*, *Coloration*, *Layout* and *Calculation*. The four categories can be accessed by clicking on the corresponding button on top of the algorithms widget (see top of Panel (C), Figure 1).

Filter: These algorithms allow to perform both selection and filtration. Each of these algorithms can either select the elements of interest or it can filter the other elements out. The default behavior of that category is to filter out all elements that are not of interest. In Figure 1 Panel (C), one can see a *scissors* icon which means that running the algorithm will filter out uninteresting elements. Clicking on that icon turns it into a *select* icon which means that elements will only be highlighted in the visualization.

Coloration: *rNAV* provides a list of color mapping algorithms. These mappings can be done on network nodes (*i.e. the RNAs*) as well as on the edges (*i.e.* the putative interactions). Among them, one can colorize the elements (nodes and edges) according to input data such as the p-value associated to the putative interactions or the type of RNA (sRNA and mRNA). In addition, the coloring can be done according to simple theoretical measures like node's degree, betweenness centrality [10] or centrality [6].

Layout: As *rNAV* uses nodelink diagrams representation of the network, we obviously need to provide graph drawing algorithms. Our software supports four layout algorithms, two of them are force directed and are called *Efficient* and *Fast* in the graphic interface and correspond to the GEM [11] and the $FM^3$ [13] algorithms. We also provide a dedicated hierarchical drawing algorithm and a basic circular drawing one.

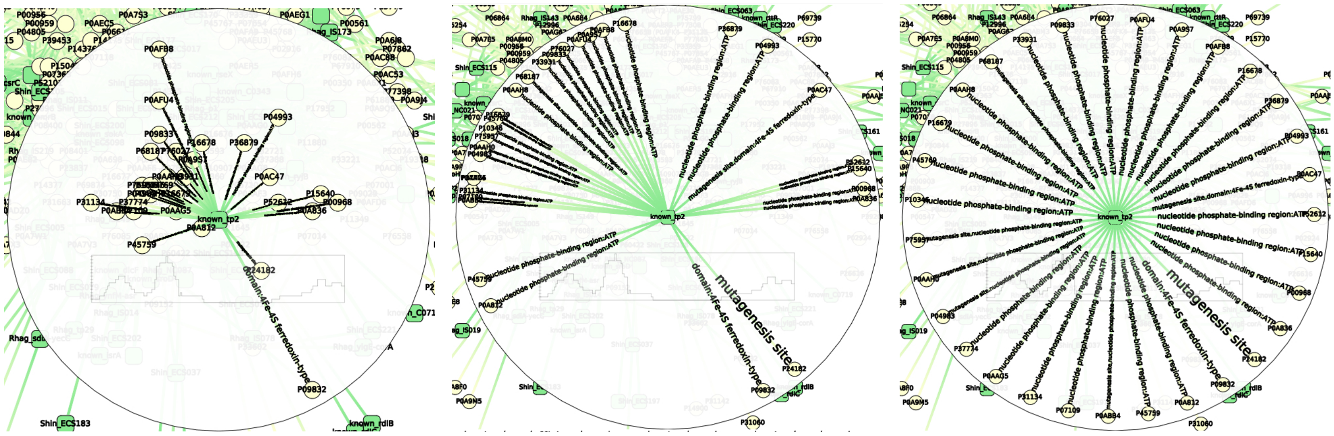
Calculation: These algorithms allow to compute simple however useful graph theoretical measures like degree, betweenness [10] or centrality [6]. In addition we also support, for each RNA, the clustering of its putative interactions. That clustering groups putative interaction according to the regions these interactions happen. To do so, we first compute the Jaccard's index [19] where the input for comparing two interactions is the region where these interactions happen. Then the MCL clustering [41] is performed to group *similar* interactions, *i.e.* according to the involved regions of the sequences.

Given the list of available algorithms, running an algorithm results in a modification of either the element color, selection, positioning or in the removal of elements. Each time an algorithm is run on a graph, we maintain user's mental map by smoothly animating the transition between the input and the ouput graph representation. For instance, when a Filter algorithm is applied to filter out elements, then we turn their colors to a fully transparent ones and finally remove them from the visualization.

To extract interesting candidates from the list all the putative interactions, the user often needs to perform several and successive filters (P5 in Table 1). *rNAV* supports the editing of algorithm pipelines as an ordered sequence of algorithms of any category (bottom of Panel (C) in Figure 1). In that sequence, the output of an algorithm is the input of the next one. The addition of an algorithm (and the ordering of the sequence) is achieved by simply dragging the algorithm from the list of available algorithms (top of Panel (C) in Figure 1) and dropping it at the right position in the pipeline (bottom of Panel (C) in Figure 1). For a reproducibility purpose, our tool also supports saving of algorithms pipelines. These saved pipelines are then reachable from the *Action list* button in the user interface (see top of Panel (C) in Figure 1).

### 3.3 Exploration Awareness and Multiple Sessions Analysis

As mentioned above, the exploration of the entire network can be fastidious and may even require an analysis over multiple sessions (P7 in Table 1). Our software has therefore to help the user to remember his previous analyses. This is achieved by a combination of features developed in *rNAV*. First of all, based on the work of Shrinivasan and van Wijk [37], we propose an *exploration tree* view (see Panel (B) of Figure 1). The root node of the tree is associated to the entire network. When running an algorithm on a -sub-network associated to a node *u* of the tree, we add a new child to *u* corresponding to the output of the algorithm. To help the user to remember the algorithms which lead to a given *state*, the user can add information to each node of the exploration tree (by default, the information is the name of the corresponding algorithm). That information can then be modified by double-clicking on the exploration tree node. Running a pipeline of algorithms creates a new branch in the tree where each node of the branch correspond to an algorithm of the pipeline.

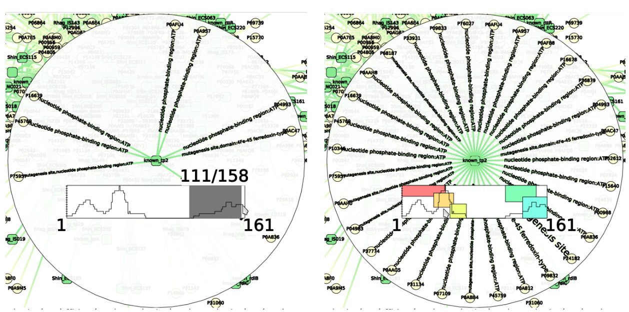


(a) (b) (c)

Figure 2: Different steps of our dedicated Bring & Go [26] interaction tool. (a) When the mouse pointer is over a RNA, an augmented display emphasizes its neighborhood. (b) Clicking on that RNA set it as focused and its neighbors are all laid at the same distance, (c) finally the neighbors are uniformly laid out on that circle while preserving the order of the neighbors to avoid edge crossings and maximize the area available to show annotations.

Then, to support multiple sessions analysis, the user can save the current state of his analysis. In particular, *rNAV* can save the network as well as all the exploration tree. During the next sessions, the user will be able to access each graph corresponding to each node of the exploration tree. Multiple sessions analysis is also eased by saving algorithms pipelines as the user can then reuse the same algorithms pipelines and their parameters as they were during a previous sessions.

### 3.4 Interaction Tools



(a) (b)

Figure 3: Selection of RNAs according to their interacting regions with our dedicated Bring & Go [26] interaction tool. Once a node of interest have been clicked, the user can select a region of interest (a) or, if the interactions have been clustered, selectable rectangles are used to show up the corresponding interacting regions (b).

First of all, *rNAV* provides classical *zoom and pan* and *box selection* interaction tools to support usual exploration of graph.

We also implemented a dedicated interaction tool to fit with the tasks of our users (P2 and P4 in Table 1). That interaction tool, inspired from the *Bring & Go* of Moscovich *et al.* [26], allows to efficiently visualize the neighborhood of a given RNA (see Figure 2). The basic of the original method is to *bring* the neighbors of a node of interest and then clicking on one of these neighbors changes the focus to that clicked node (*go* step).
In *rNAV*, the annotation information is of upmost importance, we therefore modified the layout of the neighbors to ensure all edges labels to be readable (see Figure 2). Instead of laying out the neighbors on several concentric circles indicating the relative distances, we decided to lay them out on a single one (see Figure 2.(b)). As we preserve the direction of the neighbor positions, some node overlaps can occur. These overlaps are removed during that step which lays the neighbors on the circle in a regular manner (see Figure 2.(c)). To preserve as much as possible the user mental map, we ensure during that animation that no edge crosses another. This is achieved by keeping the order of the neighbors around the focus node unchanged during the animation. Such modification of [26] maximizes the available area for drawing the annotations adjacent to a focused RNA. Still the readability of one of these annotations decreases as its length (number of characters) increases, but also as the total number of adjacent annotations increases.

Another important information is the regions of the focused RNA which interact with other RNAs (P4 in Table 1). Indeed, a region interacting with many other RNAs may be considered as an evolutionary constrained region. That interaction tool therefore supports, for each base of that focused RNA, the visualization of the number of interactions each of its bases is involved in. That information is displayed as a curve inside a new glyph we have designed. In Figure 3.(a), one can see that the focused RNA molecule is made of 161 bases (numbered from 1 to 161) and that two main regions interact with other RNAs. In case where the interactions have been clustered (see section 3.2), then the resulting clustering is displayed with that glyph. Each cluster is represented by a colored rectangle spanning the region of the corresponding interaction (in Figure 3.(b), 5 clusters of interactions have been identified). Even if the MCL algorithm produces a partition of the putative interactions, the corresponding regions can overlap. In that case, we ensure the visibility of each cluster as we reduce the height of its box by the number of overlapping clusters (see Figure 3.(b)). Moreover the coloring algorithm ensures that such boxes will be colored with different colors. The last feature of that interaction tool is the selection within the glyph itself. The user can either select neighbors of the focused RNA by using a box selection on the glyph or by simply clicking on one of the colored rectangle once the interactions have been clustered (see Figure 3). In both case the corresponding region of the RNA molecule is displayed above the curve (in Figure 3.(a) the region from base 111 to base 158 has been selected).

### 3.5 Multiple/Linked Views

To compare the results of different filters or of different filter parameters (P6 in Table 1), *rNAV* supports multiple and linked views. The visualized -sub-networks are selected by the user in the exploration tree. Using the Ctrl key and clicking on an exploration tree node, the user can add or remove the view on the corresponding -sub-network.

As mentioned above, all displayed views are linked. In particular, any interaction on a view will affect the others. Selecting elements with the box selection tool in one view will also select these elements in all the views (if present in the corresponding networks). When using the pan and zoom interaction tool to navigate within a given view, the user can focus on a particular subset of RNAs. In that case, we compute the set of RNAs of interest and automatically perform a combination of pan and zoom as defined by van Wijk and Nuij [42] to focus in the other views on the same RNAs (again if these RNAs are present in these views). Finally, any action performed with de dedicated Bring & Go interaction tool (focus, *bring* or *go*) impacts the other views as well. For instance, in Figure 1, the focus has been set on a RNA of the bottom right view and the corresponding neighborhood is highlighted in the three others.

## 4 USE CASE: ANALYSIS OF *Escherichia coli* BACTERIA

To demonstrate the usefulness of *rNAV*, we experimented the software for the analysis sRNA regulatory network of *Escherichia coli K12*. This bacteria, easily cultivable under laboratory conditions, is a bacterial model organism that has been extensively analyzed over the last decades. Whereas transcription factors are presents in this bacteria, several publications have demonstrated the crucial role carried out by sRNA regulation. An interesting hypothesis relies on the benefit gained by the cell from sRNA regulation. Moreover, such type of regulation has been recently pointed out by several publications when bacterial cells have to increase their survival behavior during a stressful situation (for a review see [39]). Recent experimental works have led to the discovery of 85 sRNAs in *Escherichia coli K12*. We collected the experimental validated sRNA data from [28] to predict, for each of them, their potential annotated mRNA targets (as parameters, we extracted regions located -150/+50 from the AUG start codon), by using our prediction protocol (described in section 2). First, the *Escherichia coli K12* sRNAs have been requested using IntaRNA versus the complete mRNAs list of *Escherichia coli K12* (4142 mRNAs) to predict potential targets (downloaded from the EMBL database, accession number: $U00096$). We generated a regulatory network with 60346 edges modeling the predictive interactions between the 85 sRNA and 4142 mRNA nodes. Considering the lack of specificity given by IntaRNA, we then further analyzed the predictions by adding enriched annotations using DAVID. At last, we exploited *rNAV* to guide the data analysis by taking into account multi-purpose types of biological constraints.

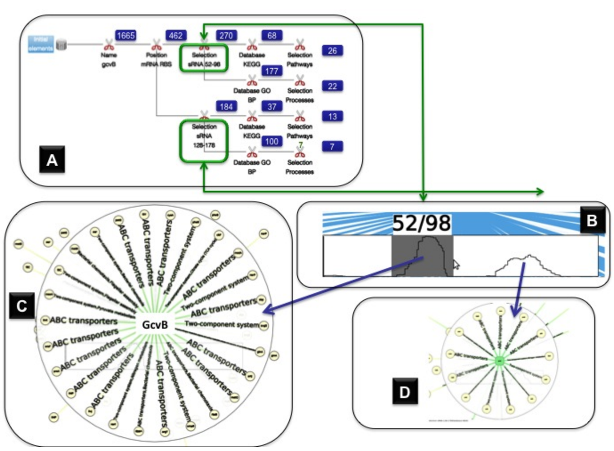



Figure 4: Illustration of the GcvB sRNA hub analysis. Panel (A) depicts the analysis process that was applied to the regulatory network, while (B)-(D) are screen captures of the different filtering steps. On Panel (A), for each filtration step, the resulting number of target candidates is shown within a blue rectangle. On panel (B), a graphical representation of the number of GcvB targets, according to their interacting localizations on the sRNA (given by the x-axis). The highest values of the curve delimit two sRNA regions that correspond to the R1 and R2 regions, proposed for the *Salmonella* GcvB sRNA. Panels (C) et (D) are computed by selecting R1 and R2 through the curve on Panel (B).

The well characterized small RNA *GcvB* was first investigated, as more than 10 targets have been experimentally validated in the *Salmonella* genome [35, 36]. A comparative analysis between a GcvB *Salmonella* mutant and a wild *Salmonella* strain have shown a variation in the expression of more than 70 genes. Regarding these results, Sharma and Storz [36] have suggested the involvement of GcvB in the regulation of more than 1% of the *Salmonella* genes and the ability to play the role of a strong pervasive regulator. A GcvB secondary structure composed of five stems and two single strand regions (namely R1 and R2) was proposed by Sharma *et al* [35]. Both regions R1 and R2 are interacting with mRNAs. For the R1 region, experimental works have shown the GcvB regulatory role with seven mRNAs involved in the peptide transport or/and the acid stress response [35, 20]. Concerning the second region (R2), three targets have been validated so far [36, 9]. In regard of these biological informations about the *Salmonella* genome and the presence of the GcvB sRNA in the *Escherichia coli K12* genome [30], the two following questions have been addressed with *rNAV*:

1. Can we transpose what is experimentally known from one genome to a relative one? (*i.e.* from *Salmonella* to *Escherichia coli*).

2. Can we expand the previous GcvB target list by exploiting biological knowledge of multiple targeting features?

To answer both questions, we have carried out the following data-driven bioinformatics approach which combines experimental *Salmonella* knowledge with predictive results compiled for *Escherichia coli K12* (Panel (A), Figure 4). First, we focused on a -sub-network by selecting the name of the sRNA of interest, GcvB in the present case, as well as its direct neighborhood. Second, we applied several combinations of filters and interactions:

- A constrained interval for the mRNA interacting region was set: sRNAs may regulate the translation of mRNAs by competing with ribsosome on its mRNA fixation region, usually ranged in an interval of positions of (-20,+10) regarding the position of the initiation codon of the mRNA.

- We then selected, using the dedicated Bring & Go interaction tool, regions of the GcvB strand and filtered out from GcvB neighborhood, mRNAs not interacting on one of these regions. The evolutionary conservation of one sRNA region interacting with multi-targets is a feature that has been already exploited. In that case, two regions were identified, the first one from the position 52 to 98 and the second one from the position 121 to 178 (see Panel (B), Figure 4). These regions correspond respectively to the R1 and R2 strand regions and have been already identified for 10 validated targets. From both of them, exploiting the specific selection tool, two branches were created in the exploration tree.

- Two additional filters were applied to filter out targets according to their annotations in the KEGG and the Gene Ontology databases. The first filter aimed at selecting pathways involved in amino acid transport and two-component system (see Panel (C), Figure 4 for the R1 region). The second one involved the Gene Ontology database by requiring specific annotations related to the same biological processes (see Panel (D), Figure 4 for the R2 region).

Exploiting this procedure, two prioritized lists of target candidates have been proposed for the two interacting regions onto GcvB (R1 and R2), with respectively 37 and 18 mRNAs. These two gene lists come from the combination of two filters (specific enriched annotations from GO and KEGG) after removing redundant candidates that were found with both databases. The filters that we have applied satisfy several constraints that may help to easily argue their selection for experimental validations. Among them, it is noteworthy that (1) the R1 and R2 regions in the *Salmonella* genome are observable on the *Escherichia coli K12* GcvB as most of the mRNA candidates are interacting on both regions (Panel (B), Figure 4) and (2) among the list of validated targets for *Salmonella*, eight were found for *Escherichia coli K12* .

A second analysis has been carried out by focusing on the *RpoS* mRNA as a starting point. The main objective was to investigate a DOR motif where a mRNA can be regulated by several sRNAs, *i.e.* when the cell needs to coordinate an answer to several stress sources. The RpoS mRNA has been reported to encode for a major stress sigma factor which is involved in stressful conditions. Furthermore 4 regulating sRNAs have been reported to base-pair the 5′ region of RpoS mRNA [23]. Using *rNAV*, we selected the RpoS sub-network and compiled, with the clustering algorithm, the groups of targets according to their interacting positions. The interaction clustering provided 3 clusters represented by colored rectangles in Panel (A) of Figure 5. We selected the UTR overlapping region cluster to interactively emphasize the corresponding interacting sRNAs (Panel (B), Figure 5). After analysing it, we noticed the presence of 3 out of the 4 experimentally validated sRNAs, as well as 3 new putative interacting sRNAs (*rydB*, *rybA* and *tp2*), for which there is no known targets [28]. Interestingly, these 3 sRNAs are presents in the 27 genome shigella-coli clade like the 4 already validated ones. This analysis helped us generate a new hypothesis regarding the Gottesman *et al.* hypothesis [12, 38] where sRNAs sharing targets are more likely to be conserved in the shigella-coli clade as they may be derived from a common sRNA ancestor. This type of analysis would not have been possible without combining biological as well as topological features through *rNAV*.

## 5 Conclusion

While RNA-Seq technologies provide an increasing number of non coding RNA sequences for different bacteria, *in silico* studies of their functional activities is still challenging. Indeed such studies require the use of bioinformatics tools dedicated to the prediction of potential mRNA targets, and these tools are known to provide a prohibitive number of false-positive candidates. In this paper, we presented rNAV, a new visualization software designed for the analysis of sRNA-mediated regulatory networks. rNAV allows experts to drive bioinformatics analysis while exploiting the complete or pertinent sub-part of the network. Its features are designed to guide the analysis by integrating multi-purposed biological knowledge in order to help experts to explore hypotheses. rNAV allows to explore large sRNA-mediated regulatory network by gradually reducing the scope of the study. Providing appropriate filtration algorithms is therefore one of the key features of rNAV. Exploiting enriched annotations, rNAV allows to identify pertinent sRNA candidates as well as reasonable putative interacting mRNAs. Using this information, bioinformaticians can then determine sRNA's functional activities to be confirmed by experimental validations.

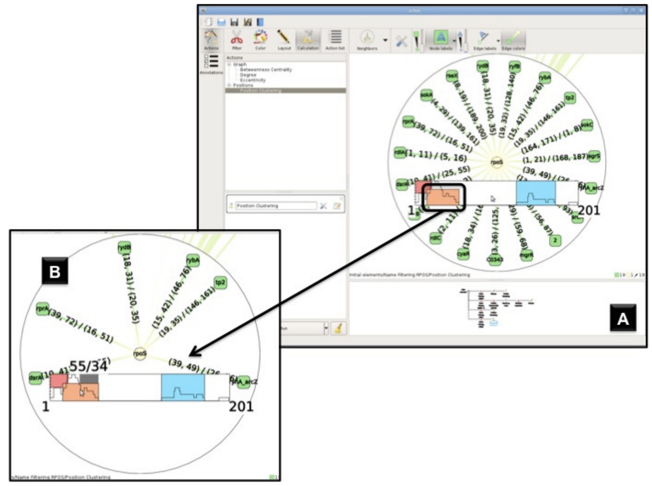



Figure 5: Illustration of the RpoS mRNA hub analysis. sRNA nodes are represented as green circles and mRNA nodes are in yellow circles. Panel (A) represents the RpoS sub-network and the visualization of the clustering algorithm for grouping potential base-pairing sRNAs. Each group are represented by a colored rectangle and one can uses this representation to carry out a selection to define a pertinent sub-network. Panel (B) depicts the sub-network of the sRNAs interacting with the 5′ region of RpoS.

rNAV also integrates a dedicated Bring & Go interaction tool to highlight a given RNA's neighborhood in the whole network but also to visually identify the evolutionary conserved interacting regions of that RNA.

We applied our software to the analysis of the *Escherichia coli K12* sRNA-mediated regulatory network to address two issues. First, we exploited biological known features for the GcvB sRNA to expand its list of mRNAs targets, in accordance to their involvement in amino acid transport and its potential pervasive regulatory function as suggested for *Salmonella*. Second, we focused on a putative mRNA hub, identified as a sigma factor, and exploit rNAV to argue the proposition of 3 new interacting sRNAs.

An interesting direction for future work is to integrate new biological constraints from future experimental research work, that is facilitated by the modular implementation of rNAV. In rNAV, we consider as input data the regulatory network together with the interacting regions and the enriched annotations. In this first version of rNAV, the prediction stages are not included. Therefore, another direction is to give more flexibility to the user and to allow the import of all these data (from a genbank or embl file), and on demand, retrieve annotations from DAVID for instance. Hence, an up-going version of rNAV will allow to calculate the graph by the combination of two stages (described in section 2) to enables the prediction of sRNA-mRNA bacterial interactions and to perform enrichments using statistical approaches.

Last direction is to support the comparison of different organisms' networks. Such comparisons can be done according to different concepts, for instance, biological knowledge (*e.g.* functional annotations) or topological information.

## Acknowledgements

This work was partially done under the EVIDEN project (ANR 2010 JCJC 0201 01), supported by the ANR (France) ; and under the MycoRNA project, PEPS CNRS/IdEx Bordeaux, 2013.

## References

[1] M. Ashburner, C. A. Ball, J. A. Blake, D. Botstein, H. Butler, J. M. Cherry, A. P. Davis, K. Dolinski, S. S. Dwight, J. T. Eppig, M. A. Har-

ris, D. P. Hill, L. Issel-Tarver, A. Kasarskis, S. Lewis, J. C. Matese, J. E. Richardson, M. Ringwald, G. M. Rubin, and G. Sherlock. Gene ontology: tool for the unification of biology. the gene ontology consortium. *Nat Genet*, 25(1):25–29, May 2000.

[2] D. Auber. *Graph Drawing Software*, chapter Tulip- A Huge Graph Visualization Framework. Springer-Verlag, 2003.

[3] J. Baumbach, T. Wittkop, K. Rademacher, S. Rahmann, K. Brinkrolf, and A. Tauch. Coryneregnet 3.0–an interactive systems biology platform for the analysis of gene regulatory networks in corynebacteria and escherichia coli. *J Biotechnol*, 129(2):279–289, Apr 2007.

[4] C. L. Beisel and G. Storz. Base pairing small rnas and their roles in global regulatory networks. *FEMS Microbiol Rev*, 34(5):866–882, Sep 2010.

[5] S. I. Berger, R. Iyengar, and A. Ma'ayan. Avis: Ajax viewer of interactive signaling networks. *Bioinformatics*, 23(20):2803–2805, Oct 2007.

[6] U. Brandes and D. Wagner. *Visone – Analysis and Visualization of Social Networks*, pages 321–340. Springer-Verlag, 2003.

[7] K. R. Brown, D. Otasek, M. Ali, M. J. McGuffin, W. Xie, B. Devani, I. L. v. Toch, and I. Jurisica. Navigator: Network analysis, visualization and graphing toronto. *Bioinformatics*, 25(24):3327–3329, Dec 2009.

[8] A. Busch, A. S. Richter, and R. Backofen. Intarna: efficient prediction of bacterial srna targets incorporating target site accessibility and seed regions. *Bioinformatics*, 24(24):2849–2856, Dec 2008.

[9] A. Coornaert, C. Chiaruttini, M. Springer, and M. Guillier. Post-transcriptional control of the escherichia coli phoq-phop two-component system by multiple srnas involves a novel pairing region of gcvb. *PLoS Genet*, 9(1):e1003156, Jan 2013.

[10] L. Freeman. A set of measures of centrality based on betweenness. *Sociometry*, 40(1):35–41, March 1977.

[11] T. M. J. Fruchterman and E. M. Reingold. Graph drawing by force-directed placement. In *Software-Practice and Experience*, volume 21(11), pages 1129–1164. SoftwarePractice & Experience, nov 1991.

[12] S. Gottesman and G. Storz. Bacterial small rna regulators: versatile roles and rapidly evolving variations. *Cold Spring Harb Perspect Biol*, 3(12), Dec 2011.

[13] S. Hachul and M. J?nger. Drawing large graphs with a potential-field-based multilevel algorithm. In *Proc. Graph Drawing 2004 (GD'04)*, pages 285–295, 2004.

[14] Z. Hu, E. S. Snitkin, and C. DeLisi. Visant: an integrative framework for networks in systems biology. *Brief Bioinform*, 9(4):317–325, Jul 2008.

[15] D. W. Huang, B. T. Sherman, and R. A. Lempicki. Bioinformatics enrichment tools: paths toward the comprehensive functional analysis of large gene lists. *Nucleic Acids Res*, 37(1):1–13, Jan 2009.

[16] D. W. Huang, B. T. Sherman, and R. A. Lempicki. Systematic and integrative analysis of large gene lists using david bioinformatics resources. *Nat Protoc*, 4(1):44–57, 2009.

[17] D. W. Huang, B. T. Sherman, Q. Tan, J. Kir, D. Liu, D. Bryant, Y. Guo, R. Stephens, M. W. Baseler, H. C. Lane, and R. A. Lempicki. David bioinformatics resources: expanded annotation database and novel algorithms to better extract biology from large gene lists. *Nucleic Acids Res*, 35(Web Server issue):W169–W175, Jul 2007.

[18] F. Iragne, M. Nikolski, B. Mathieu, D. Auber, and D. Sherman. Proviz: protein interaction visualization and exploration. *Bioinformatics*, 21(2):272–274, 2005.

[19] P. Jaccard. Distribution de la flore alpine dans la bassin de dranses et dans quelques régions voisines. *Bulletin de la Société Vaudoise des Sciences Naturelles*, 37:241–272, 1901.

[20] Y. Jin, R. M. Watt, A. Danchin, and J.-d. Huang. Small noncoding rna gcvb is a novel regulator of acid resistance in escherichia coli. *BMC Genomics*, 10:165, 2009.

[21] M. Kanehisa, S. Goto, Y. Sato, M. Furumichi, and M. Tanabe. Kegg for integration and interpretation of large-scale molecular data sets. *Nucleic Acids Res*, 40(Database issue):D109–D114, Jan 2012.

[22] A. Lysenko, A. Lysenko, M. M. Hindle, J. Taubert, M. Saqi, and C. J. Rawlings. Data integration for plant genomics–exemplars from the integration of arabidopsis thaliana databases. *Brief Bioinform*, 10(6):676–693, Nov 2009.

[23] P. Mandin and S. Gottesman. Integrating anaerobic/aerobic sensing and the general stress response through the arcz small rna. *EMBO J*, 29(18):3094–3107, Sep 2010.

[24] M. J. McGuffin and I. Jurisica. Interaction techniques for selecting and manipulating subgraphs in network visualizations. *IEEE Trans Vis Comput Graph*, 15(6):937–944, 2009.

[25] S. R. Modi, D. M. Camacho, M. A. Kohanski, G. C. Walker, and J. J. Collins. Functional characterization of bacterial srnas using a network biology approach. *Proc Natl Acad Sci U S A*, 108(37):15522–15527, Sep 2011.

[26] T. Moscovich, F. Chevalier, N. Henry, E. Pietriga, and J.-D. Fekete. Topology-Aware Navigation in Large Networks. In A. Press, editor, *SIGCHI conference on Human Factors in computing systems*, pages 2319–2328, Boston, États-Unis, 2009. ACM.

[27] C. Pastrello, D. Otasek, K. Fortney, G. Agapito, M. Cannataro, E. Shirdel, and I. Jurisica. Visual data mining of biological networks: One size does not fit all. *PLoS Comput Biol*, 9(1):e1002833, 01 2013.

[28] A. Peer and H. Margalit. Accessibility and evolutionary conservation mark bacterial small-rna target-binding regions. *J Bacteriol*, 193(7):1690–1701, Apr 2011.

[29] R. Pesch, A. Lysenko, M. Hindle, K. Hassani-Pak, R. Thiele, C. Rawlings, J. Khler, and J. Taubert. Graph-based sequence annotation using a data integration approach. *J Integr Bioinform*, 5(2), 2008.

[30] S. C. Pulvermacher, L. T. Stauffer, and G. V. Stauffer. Role of the srna gcvb in regulation of cyca in escherichia coli. *Microbiology*, 155(Pt 1):106–114, Jan 2009.

[31] P. Romby and E. Charpentier. An overview of rnas with regulatory functions in gram-positive bacteria. *Cell Mol Life Sci*, 67(2):217–237, Jan 2010.

[32] P. Romby and E. G. H. Wagner. Exploring the complex world of rna regulation. *Biol Cell*, 100(1):e1–e3, Jan 2008.

[33] M. Secrier, G. A. Pavlopoulos, J. Aerts, and R. Schneider. Arena3d: visualizing time-driven phenotypic differences in biological systems. *BMC Bioinformatics*, 13:45, 2012.

[34] J. A. Shapiro. Revisiting the central dogma in the 21st century. *Ann N Y Acad Sci*, 1178:6–28, Oct 2009.

[35] C. M. Sharma, F. Darfeuille, T. H. Plantinga, and J. Vogel. A small rna regulates multiple abc transporter mrnas by targeting c/a-rich elements inside and upstream of ribosome-binding sites. *Genes Dev*, 21(21):2804–2817, Nov 2007.

[36] C. M. Sharma, K. Papenfort, S. R. Pernitzsch, H.-J. Mollenkopf, J. C. D. Hinton, and J. Vogel. Pervasive post-transcriptional control of genes involved in amino acid metabolism by the hfq-dependent gcvb small rna. *Mol Microbiol*, 81(5):1144–1165, Sep 2011.

[37] Y. B. Shrinivasan and J. J. van Wijk. Supporting Exploration Awareness in Information Visualization. *IEEE Computer Graphics and Applications*, 29(5):34–43, 2009.

[38] E. Skippington and M. A. Ragan. Evolutionary dynamics of small rnas in 27 escherichia coli and shigella genomes. *Genome Biol Evol*, 4(3):330–345, 2012.

[39] G. Storz, J. Vogel, and K. M. Wassarman. Regulation by small rnas in bacteria: Expanding frontiers. *Mol Cell*, 43(6):880–891, Sep 2011.

[40] A. Toledo-Arana, F. Repoila, and P. Cossart. Small noncoding rnas controlling pathogenesis. *Curr Opin Microbiol*, 10(2):182–188, Apr 2007.

[41] S. van Dongen. *Graph Clustering by Flow Simulation*. PhD thesis, Universiteit Utrecht, 2000.

[42] J. J. van Wijk and W. A. A. Nuij. Smooth and efficient zooming and panning. In *Proceedings of the Ninth annual IEEE conference on Information visualization*, INFOVIS'03, pages 15–22, Washington, DC, USA, 2003. IEEE Computer Society.

[43] J. Vogel and E. G. H. Wagner. Target identification of small noncoding rnas in bacteria. *Curr Opin Microbiol*, 10(3):262–270, Jun 2007.

[44] L. S. Waters and G. Storz. Regulatory rnas in bacteria. *Cell*, 136(4):615–628, Feb 2009.