Modules of human micro-RNA co-target network

Mahashweta Basu\textsuperscript{1,\dagger}, Nitai P. Bhattacharyya\textsuperscript{2}, P. K. Mohanty\textsuperscript{1}

\textsuperscript{1}Theoretical Condensed Matter Physics Division, Saha Institute of Nuclear Physics, 1/AF Bidhan Nagar, Kolkata 700064, India.
\textsuperscript{2}Crystallography and Molecular Biology Division, Saha Institute of Nuclear Physics, 1/AF Bidhan Nagar, Kolkata 700064, India.

E-mail: \textsuperscript{\dagger}mahashweta.basu@saha.ac.in

Abstract. Human micro RNAs (miRNAs) target about 90\% of the coding genes and form a complex regulatory network. We study the community structure of the miRNA co-target network considering miRNAs as the nodes which are connected by weighted links. The weight of link that connects a pair of miRNAs denote the total number of common transcripts targeted by that pair. We argue that the network consists of about 74 modules, quite similar to the components (or clusters) obtained earlier [Online J Bioinformatics, 10,280], indicating that the components of the miRNA co-target network are self organized in a way to maximize the modularity.

1. Introduction

Micro RNAs are a class of small single stranded non-coding RNAs, about 20 to 22 base long, which interfere with the translation of messenger RNAs (mRNAs) by binding to their 3' untranslated regions (UTR) \cite{1}. Several computational methods \cite{2} have been developed for predicting the mRNA transcripts which are possible targets of a particular miRNA. For example, 711 nucleotide sequences are predicted as miRNAs \cite{3} of human; their possible targets, (34525 in total) are listed in the mirBASE database \cite{4}. It has been proposed on the basis of theoretical analysis that as large as 90\% human genes are targets of miRNA \cite{5}. Regulation of coding genes by miRNAs in combination are also experimentally validated \cite{6}.

The abundance of miRNA and their targets provide enormous combinatorial possibilities for regulation. Combinatorial regulation of genes by transcription factor (TF) and miRNAs provides higher complex programs \cite{7}. Recently, taking TFs as important mediators of miRNA-initiated regulatory effects, it was shown \cite{8} that the underlying network is significantly associated with multicellular organismal development, cell development and cell-cell signaling. Combinatorial effect of miRNA modules \cite{9} has been observed in tumor tissues or cell lines. This observation suggests a combinatorial effect of the module associated miRNAs on target gene regulation in selective tumor tissues or cell lines. Synergistic network \cite{10} of miRNAs reveals that miRNA modules associated with diseases are significantly different from modules of miRNAs that does not involve in disease. Possibility of co-regulation of two or more miRNAs in context of gene expressions and relevant biological functions is, however, least explored.

Recently Mookherjee \textit{et. al.} \cite{11} have analyzed the miRNA co-target network (MCN) of \textit{Homo sapiens}, which indicate that several group of miRNAs (so called \textit{clusters}) provide most essential regulations. This topological analysis of miRNA network revealed that about 70 clusters
of miRNAs co-target the genes, which are involved in specific pathways. For several clusters, all miRNAs belonging to the cluster are found to be maximally expressed in a specific tissue. Further studies [12], indicate that the clusters are also disease specific. Reorganizing miRNAs into such groups (clusters) helps in identifying cooperative activity of miRNAs. In fact, from these analysis one can predict that, “if one miRNA from a particular cluster is involved in a specific biological pathway or cellular function, the other miRNAs belonging to the same cluster are likely to be involved in the same disease, pathway or function”.

Detection of communities, groups, components or clusters have been a focus of recent interest in context of complex networks. Networks like the world wide web [13], the metabolic network [14], the social network [15], protein protein interaction network [16] etc. do possess community structures, meaning the vertices tend to divide into groups, with dense connections within the groups and sparse connection existing among the groups. These communities act as the functional units of the network; for example ‘ATP synthesis’, ‘DNA processing’, and ‘cell cycle control’ are well known [17] functional modules of yeast protein-protein interaction network. Evidently, the functional properties of an entire network is quite different from their properties at community level.

In this article, we study the community structures (modules) of miRNA co-target network of human and compare them with the components (clusters) of miRNAs obtained earlier [11]. Since the components of a network are the only disjoint subgraphs, it is expected that the community structures can be better represented by the modules. This is explained schematically in Fig. 1, where the network has 3 components and 6 modules.

In section 2 we briefly review the relevant features of the miRNA co-target network of human and its components (clusters). In section 3 we apply the modularization method introduced by Newmann [18] to analyze this miRNA co-target network and compare the resulting modules with the clusters obtained earlier[11]. Finally, conclusions are given in section 4.

Figure 1. Component versus modules : Components (or clusters) are disjoint sub-graphs of a network (they do not have any common link), whereas modules may have relatively fewer connections among them. The above network has 3 components namely (a), (b) and (c) with each component consisting of 1, 3 and 2 modules respectively. Component (b) has three modules consisting of 4, 9 and 5 vertices, similarly component (c) consists of two modules comprising of 3 and 5 vertices.
2. Clusters of miRNAs in the co-target network

Let us briefly revisit the main ideas and results of Ref. [11] to understand the construction, topology and components of human miRNA co-target network consisting of 711 miRNAs and their 34525 predicted targets obtained from the miRBase database (http://microrna.sanger.ac.uk/, version 10). For convenience, miRNAs are given arbitrary identification number \( m = 1, 2, \ldots, i, \ldots, M \), where \( M = 711 \). Further, the miRNA co-target network was constructed by considering miRNAs as the nodes and joining every pair of miRNAs having one or more common targets with a link. The total number of co-targeted transcripts \( C_{ij} \) of miRNAs \( i \) and \( j \) is taken to be the weight of the corresponding link. Clearly, the resulting adjacency matrix \( C \) is symmetric (with diagonal elements \( C_{ii} = 0 \)).

Mookherjee et. al. [11] have proposed an elegant method for finding the clusters of miRNAs. Since substantially large number of miRNA pairs have only few co-targets, the links between them have small weights, and can be erased to obtain a simplified network. Let \( N_q \) be the number of components of the network when all the links having weight less than \( q \) are erased. Thus, the network breaks into smaller disjoint subgraphs (components) with rate \( dN_q/dq \), which is maximum at \( q = q^* \). It was argued that among all the subgraphs of the co-target network obtained at \( q^* \), the largest one is the most important; miRNAs belonging to this subgraph are found to down regulate expression of genes involved in several genetic diseases.

To be specific, the human miRNA co-target network breaks into \( N_q = 166 \) subgraphs at \( q = 103 \), where the largest subgraph called \( \mathcal{G} \) contain 477 miRNAs. To determine how miRNAs are organized within the subgraph \( \mathcal{G} \), \( q \) is increased further. At \( q = 160 \) the subgraph \( \mathcal{G} \) breaks up into 70 small clusters (the subgraphs having two or more miRNAs) and 147 independent miRNAs. Out of total 70, 18 clusters arise from the seed sequence\(^1\) similarities and 11 clusters are organized into the same genomic region (5 inter-genomic clusters also show seed sequence similarities). Most of the clusters are found to be either pathways, tissues and/or diseases specific.

In the following section we aim at investigating the modular structure of miRNA co-target network. Figure 1 schematically describes, why a network is better represented by its modules than its components (disjoint subgraphs).

3. Modular structure of miRNA network

The identification of community structure is one of the many challenging problems in various scientific field. A large variety of community detection techniques have been developed based on centrality measures, link density, percolation theory etc. Recently, Newman et. al.[18] proposed a method of finding community structure of a network based on maximization of the modularity. This method is further generalized to include weighted networks[19].

The most obvious way of finding groups in a network is to minimize the number of edges connecting the groups. Simply rearranging the network, such that only few edges exist between the communities, is not enough. Rather one must rearrange it in a way that communities are connected with fewer than expected edges. One can associate a score called modularity \( Q \) [20] for each possible partition of a network. \( Q \) is defined up to a multiplicative factor as the number of edges present within the groups minus the expected number in an equivalent random network. Since positive values of \( Q \) indicates possible presence of community structure, one need to look for a partition for which the modularity is preferably large and positive.

A good partition of a network can be obtained by maximizing the modularity index \( Q \) defined as follows. Let us consider a network with \( n \) vertices labeled by \( i = 1, \ldots, n \), and \( m \) links. The corresponding adjacency matrix is \( A_{ij} \). Let the degree of each vertex \( i \) is \( k_i = \sum_j A_{ij} \), thus \( m = \frac{1}{2} \sum_i k_i \). If the network is to be partitioned into two groups, one associates a quantity \( s_i \)

\(^1\) The nucleotides 2 - 7 of the miRNAs are called seed sequences.
Figure 2. When all the links having weight less than \( q \) are erased, the miRNA co-target network breaks into \( N_q \) distinct components comprising of \( \mu_q \) modules. The main figure compares \( \mu_q \) (solid line) with the \( N_q \) (circles). The inset shows that the \( \frac{d\mu_q}{dq} \) is maximum at \( q = 103 \), which is same as the value obtained from \( \frac{dN_q}{dq} \) earlier [11].

which takes a value \( s_i = +1(-1) \) if vertex \( i \) belongs to group 1 (group 2).

Correspondingly the modularity is given by

\[
Q = \frac{1}{4m} \sum_{ij} \left( A_{ij} - \frac{k_i k_j}{2m} \right) \left( 1 + s_i s_j \right),
\]

(1)

where \( k_i k_j / 2m \) is the expected number of links between \( i \) and \( j \), if edges were placed at random. The term \( (1 + s_i s_j) \) is 0 (1) if vertices \( i \) and \( j \) belong to different (same) group; this assures that \( Q \) is maximum when two groups are connected by smaller than expected number of links. In the following we apply this procedure to obtain modular structure of MCN.

MCN is a undirected weighted network, where the weight of the link \( C_{ij} \) corresponds to the number of transcripts being co-targeted by the concerned pair of miRNAs \( i \) and \( j \). The diagonal elements are taken \( C_{ii} = 0 \), as usual. It has been pointed out in Ref. [11] that the weights vary widely between 1 to 1253, indicating that most of the links with small weights can be erased to obtain a simpler network. However, the connectivity of the network changes when links having weight less than a predefined value \( q \) are erased. Taking the adjacency matrix \( C^q \), defines as

\[
C^q_{ij} = \begin{cases} 
1 & \text{if } C_{ij} > q \\
0 & \text{otherwise}
\end{cases}
\]

(2)

Mookherjee et. al. [11] have calculated the number of components \( N_q \) by varying \( q \). Since, this adjacency matrix \( C^q \) is unweighted (as it keeps the information of connectivity ignoring the actual weights) one can apply the idea of modularity maximization[18] to detect the communities or modules present there. Let \( \mu_q \) be the total number of modules of \( C^q \). Clearly \( \mu_q \geq N_q \), as each component can either have one module, i.e. itself, or it can break into two or more modules. In Fig. 2 we have compared \( \mu_q \), obtained from modularization methods, with the components \( N_q \) obtained earlier. It is evident that \( \mu_q \approx N_q \); a negligible small positive difference is not visible in the figure. This brings us to conclude that the components of the network are self organized in a way that modularity (given by Eq. (1)) is maximized.

We also find that \( \frac{d\mu_q}{dq} \) is maximum at \( q = 103 \), which is the value obtained from \( \frac{dN_q}{dq} \) earlier [11]. Now let us have a closer look at the size of the components and that of the modules
Figure 3. Components of $\mathcal{G}$ (which has 477 miRNAs) : Only the clusters (components of size larger than 1) are shown. Each of the large clusters with size (7, 8, 9, 14, 16, 31, 47) appear once. The other 63 clusters comprises of (2; 29), (3; 21), (4; 2), (5; 7), (6; 2), (11; 2), where $(m; n)$ denotes that cluster of size $m$ appears $n$ times.

| Size   | 2   | 3   | 4   | 5   | 6   | 7   | 8   | 9   | 10  | 11  | 12  | 13  | 14  | 15  | 16  | 17  |
|--------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| No. of clusters | 24  | 1   | 2   | 5   | 6   | 7   | 8   | 9   | 10  | 11  | 0   | 0   | 0   | 0   | 0   | 0   |
| No. of modules   | 26  | 6   | 2   | 3   | 5   | 1   | 4   | 1   | 7   | 2   | 2   | 2   | 2   | 2   | 1   | 1   | 1   |

Table 1. Comparison of number of modules of the miRNA network with the number of components, at $q = q^* = 103$.

obtained at $q^* = 103$. This is listed in Table-I. Note, that the modularity maximization algorithm organizes the network into several small modules and few moderate size modules as 26,37,57,98,101. Whereas in terms of components the network breaks into few clusters of small sizes ($e.g.$ 2, 3, 4, 5, 7) along with a giant cluster ($\mathcal{G}$) of size 477 [11]. Evidently at $q^* = 103$, MCN has one distinctly large component containing 477 miRNAs, compared to the moderate size modules those appear with competitive sizes (101 and 98). The largest component must have been broken into these smaller modules. Thus, as far as ‘identifying a large set of relevant miRNAs’ (one like $\mathcal{G}$) is concerned, one can reliably consider the component $\mathcal{G}$ as the optimal set of miRNAs, which co-regulate the gene expressions. Further, to understand how miRNAs are organized within $\mathcal{G}$, we calculate its modules by taking $q = 160$, which is the same value of $q$ used in [11], to obtain the clusters (in total 70). All the components of $\mathcal{G}$ having two or more miRNAs (referred to as clusters), are shown in Fig. 3 in decreasing order of their sizes. The first five, named as (a) to (e) have 47, 31, 16, 14 and 9 miRNAs respectively.

It would be interesting to look at the community structure of $\mathcal{G}$ at this value of $q = 160$. Using the modularization algorithm [18], we find that $\mathcal{G}$ contains 72 modules (total 330 miRNAs) and 147 single miRNAs. Whereas in terms of components, $\mathcal{G}$ had 70 clusters (total 330 miRNAs) and 147 independent miRNAs [11]. The detailed study of modules reveals that only two of the 70 clusters are broken into smaller modules : cluster (a) in Fig. 3 with 47 miRNA, has two modules $a_1$ and $a_2$ of size 34 and 13 respectively, cluster (b) with 31 miRNAs, breaks into two modules $b_1$ (22 miRNAs) $b_2$ (9 miRNAs). Such modular structures of (a) and (b) were not apparent in Fig. 3; we redraw these graphs keeping all miRNAs in same module close to each other. The
Figure 4. The cluster (a) and (b) of Fig. 3 are redrawn to visualize the the community structure obtained through modularization. Clearly (a) which contains 47 miRNAs has two modules $a_1$ (34 miRNAs) and $a_2$ (13 miRNAs), and (b) has two modules $b_1$ (22 miRNAs) and $b_2$ (9 miRNAs).

resulting graph (Fig. 4 (a) and (b)) clearly show the existence of modular structures.

In summary, the community structure in these networks are very similar to the components (or clusters) obtained earlier in [11]. Only few large components show further small sub-structures, indicating that the existing components of MCN are already optimally modularized. Implications of these results will be discussed in section 4.

Few comments are in order. It is quite evident that cluster (c) in Fig. 3, containing 16 miRNAs, might have sub-structures of size 11 and 5, which could not be obtained when the modularization algorithm is applied to the un-weighted graph $G$ containing 477 miRNAs. In this analysis, the actual weights were ignored, i.e., all links having weight more than 160 are considered identical irrespective of their actual weight. When we keep these weights and use the modified version of the algorithm [19], that works for weighted networks, the cluster (c) shows the predicted substructures. In addition, some other modules, such as $a_2$ which has 34 miRNAs also show further sub-structures of size 30 and 4. These four nodes, turns out to be those shown in the left side of $a_2$ in Fig. 4.

It appears that Newman’s algorithm, both for un-weighted and weighted network, provides only the sub-structures of large components. This is because, modularity of the network is inversely proportional to the total number of links (see Eq. (1)). Thus, the total modularity of a network with many components is not substantially altered by re-structuring the small components into smaller sub-structures. It is only, the re-structuring of larger components which can change the modularity appreciably. To overcome this difficulty, one must find modular structures of individual components, instead of looking at the community structure of the whole network.

4. Conclusion
To our surprise, the community structure of human miRNA co-target networks is very similar to the existing components or clusters. Only few large components show smaller sub-structures.
Most of the components do not show any further substructures, indicating that the miRNA co-target network inherently consists of optimally modularized structures. It is quite possible that, during the evolution of miRNAs, first the modular structures are formed, optimized and then they join with other modules to provide essential regulation for complex life structures. Further study in these directions is required to verify such hypothesis.

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