Systems Biology Across Scales: A Personal View
XII. Intra-cellular Systems II: Metabolism and Modularity

Sitabhra Sinha
IMSc Chennai
Intra-cellular biochemical networks

- **Metabolic networks**
  Nodes: metabolites (substrates & products of metabolism)
  Links: chemical reactions (directed)

- **Genetic regulatory networks**
  Nodes: Genes & Proteins
  Links: regulatory interactions (directed)

- **Protein-Protein interaction network**
  Nodes: Proteins
  Links: physical binding and formation of protein complex (undirected)

- **Signaling network**
  Nodes: Signaling molecules e.g., kinase, cyclicAMP, Ca
  Links: chemical reactions (directed)
Metabolism

Chemical process through which cells break down nutrients to generate energy and/or into usable building blocks (catabolic metabolism) and then reassemble them using energy to form biological molecules necessary for the cell (anabolic metabolism).

Uses sequence of chemical reactions (pathways) to convert substrates (initial inputs) successively into useful products. Reactions are aided by enzymes.

Metabolic network: The set of all reactions in all pathways
Representing Metabolic Networks

Tripartite directed network

Metabolites → Reactions → Enzymes

Projection to only metabolites

KEGG
Citric acid cycle

Also known as Tricarboxylic Acid (TCA) or Krebs cycle is a series of enzyme-catalysed chemical reactions lying at the heart of aerobic metabolism.

Involved in the breakdown of all 3 major food groups: carbohydrates, lipids and proteins.
Graphical representation of metabolic networks

(a) 1 glucose 6-phosphate (G6P) + 1 NADP⁺ → 1 6-phosphoglucono δ-lactone (6PGL) + 1 NADPH
1 6-phosphoglucono δ-lactone + 1 H₂O → 1 6-phosphogluconate (6PG)
1 6-phosphogluconate + 1 NADP⁺ → 1 ribulose 5-phosphate (R5P) + 1 NADPH
1 ribulose 5-phosphate

(b) Substrate graph

(c) Reaction graph

Scale-free nature of degree distribution of metabolic networks

A portion of the WIT database for E. coli.

Nodes are substrates & products, linked by enzyme-substrate complexes (black boxes)

Organisms from all three domains of life are **scale-free** networks!

Modular nature of metabolic networks


Metabolic network of *E. coli*
(N=473, L =674).

Each circle represents a module and is colored according to the KEGG pathway classification of the metabolites it contains.
**Modular Networks**: dense connections *within* certain sub-networks (*modules*) & relatively few connections *between* modules

**Modules**: A *mesoscopic* organizational principle of networks

Going beyond *motifs* but more detailed than *global* description (L, C etc.)

---

Kim & Park, WIREs Syst Biol & Med, 2010
Modular Biology (Hartwell et al, Nature 1999)

Functional modules as a critical level of biological organization

Modules in biological networks are often associated with specific functions

Problem:
Given a network, how do we find the modules (communities) into which it can be divided?
Community Detection in Networks

Also referred to as Graph Partitioning or Module Determination

How to divide the nodes of a network into several groups such that nodes in each group are densely or strongly inter-connected

E.g., it is clear that node clusters I: \{A,B,C,D,E\} and II: \{F,G,H,I,J\} constitute two separate groups that are highly intra-connected but has only a single link connecting the two groups

The corresponding adjacency matrix will have an almost block-diagonal form – the two blocks corresponding to node clusters I & II

However for large networks the modular character may not be visually apparent – and adjacency matrices need to be partitioned
Graph partitioning

A classic problem in computer science from 1960s

How to divide the nodes of a network into a given number of non-overlapping groups of given sizes such that the number of edges between groups is minimized?

A generalization of this problem,

How to divide the nodes into several groups such that most links are within groups and few links are between groups

referred to as

Community detection

How we define “most” and “few” can vary from one algorithm to another
Spectral partitioning

Consider a network of $N$ nodes and $L$ links

Aim: to divide the $N$ nodes into 2 groups (Groups A and B, say) to reduce the cut size (number of links between the two groups)

$$R = \frac{1}{2} \sum_{ij} A_{ij}$$ such that $i$ and $j$ belong to different groups

Partitioning into more than 2 groups can be done by repeated bisection

For each node, a label $s = \{-1, +1\}$ is defined

$s_i = +1$ if node $i$ belongs to group A, $s_i = -1$ if $i$ belongs to group B

Thus

$$\frac{1}{2} (1 - s_i s_j) = 1$$ if $i$ & $j$ are in different groups,

$$= 0$$ if $i$ & $j$ are in same group

$$\Rightarrow R = \frac{1}{4} \sum_{ij} A_{ij} (1 - s_i s_j) = \frac{1}{4} \sum_{ij} (k_i \delta_{ij} - A_{ij}) s_i s_j$$

$$\Rightarrow R = \frac{1}{4} \sum_{ij} L_{ij} s_i s_j$$ where $L = D - A$ is the Laplacian matrix

In matrix notation $R = \frac{1}{4} s^T L s$ where $s = \{s_1, s_2, \ldots, s_N\}$

Goal of Partitioning: To find $s$ that minimizes $R$ given $L$
Partitioning as minimization

If \( s_i \) were allowed to take any possible value, then differentiation gives the optimum.

But \( s_i \) are restricted to \( \{-1, +1\} \Rightarrow \) difficult problem.

\( s \) can be seen as a vector that points to any one of the \( 2^N \) vertices of N-dimensional hypercube.

Possible approximate solution:
Allow \( s_i \) to take any value subject to the constraints that

(i) \( \sum_i s_i^2 = N \Rightarrow s \) is a vector in N-dimensional unit hypersphere.

(ii) \( \sum_i s_i = N_A - N_B \) where \( N_A, N_B \) are the sizes of the two groups.

In matrix notation \( I^T s = N_A - N_B \).

The minimization problem can now be solved as

\[
\frac{\partial}{\partial s_i} \left[ \sum_{jk} L_{jk} s_j s_k + \lambda \left( N - \sum_j s_j^2 \right) + 2\mu \left( [N_A - N_B] - \sum_j s_j \right) \right] = 0
\]

where \( \lambda, \mu \) are Lagrange multipliers for enforcing the constraints.

\( \Rightarrow \sum_j L_{ij} s_j = \lambda \ s_i + \mu \quad \text{In matrix notation, } L \ s = \lambda \ s + \mu \quad 1 \)
Partitioning using the Laplacian spectrum

Multiplying $L s = \lambda s + \mu I$ by $I^T$ on the left we get:

$$\lambda [N_A - N_B] + \mu N = 0 \Rightarrow \frac{\mu}{\lambda} = -\frac{[N_A - N_B]}{N}$$

Using $I^T s = N_A - N_B$ and $I^T L = 0$ ($I$ is eigenvector of $L$ with eigenvalue 0)

Defining a new vector $x = s + (\mu/\lambda) I = s - I[N_A - N_B]/N$

$$L x = L (s + (\mu/\lambda) I) = L s = \lambda s + \mu I = \lambda x$$

Thus $x$ is an eigenvector of the Laplacian

But which eigenvector?

The one that gives the smallest value of cut size $R$

We can’t choose $I=\{1,1,\ldots,1\}$ as it is orthogonal to $x$ because $I^T x = 0$

Note that cut size is proportional to the eigenvalue $\lambda$

as $R = (1/4) s^T L s = (1/4) x^T L x = (1/4) \lambda x^T x = \lambda [N_A N_B]/N$

Thus we have to choose the eigenvector corresponding to the lowest non-zero eigenvalue (smallest eigenvalue of $L$ is 0 with eigenvector $I$)

Finally, optimal partition $s$ is obtained from $s = x + I[N_A - N_B]/N$
For the actual network, the optimal partition $s$ is subject to the additional constraint that (i) $s_i = +1$ or $-1$, and (ii) exactly $N_A$ of the components are $+1$ and $N_B$ are $-1$.

Thus, we need to choose $s$ as close as possible to ideal value subject to the constraints $\Rightarrow$ maximize the vector length, i.e.,

$$s^T s = s^T (x + 1 \cdot \frac{N_A - N_B}{N}) = \sum_i s_i \left(x_i + \frac{N_A - N_B}{N}\right)$$

by assigning $s_i = +1$ for the nodes corresponding to the $N_A$ largest (most positive) values of $x$, i.e., the components of the eigenvector of the lowest non-zero eigenvalue of $L$, and, $s_i = -1$ to the remaining $N_B$ nodes.

Note: If $N_A \neq N_B$, we can either choose (i) $N_A$ elements to be $+1$ ($N_B$ elements $-1$) or (ii) $N_A$ elements to be $-1$ ($N_B$ elements $+1$)

The one having lower cut size is the optimal partition.
Community detection

How to quantify the degree of modularity for a given partitioning of a network into communities? Is there a distinction between links within a module and that between a module and the rest?

One suggested measure:

\[ Q = \frac{1}{2L} \sum_{i,j} \left[ A_{ij} \frac{k_i k_j}{2L} \right] \delta_{c_i c_j} \]

=1 if nodes are in same community

probability of an edge betn 2 nodes proportional to their degrees

A: Adjacency matrix
L: Total number of links
\( k_i \): degree of \( i \)-th node
\( c_i \): label of module to which \( i \)-th node belongs

For a random network, \( Q = 0 \)
i.e., the connection density within a module is no different from that anywhere else in the network
Community detection

For directed & weighted networks:

\[ Q^W = \frac{1}{L^W} \sum_{i,j} \left( W_{ij} - \frac{s_i^{\text{in}} s_j^{\text{out}}}{L^W} \right) \delta_{c_i c_j} \quad (L^W = \sum_{i,j} W_{ij}) \]

**W:** Weight matrix

\( s_i \): strength of \( i \)-th node

Modules determined through a generalization of the spectral method (Leicht & Newman, 2008)

Calculate eigenvector corresponding to largest +ve eigenvalue of symmetrized modularity matrix \( B + B^T \) where

\[ B_{ij} = W_{ij} - \left[ s_i^{\text{in}} s_j^{\text{out}} / L^W \right] \]

and then assign communities based on the signs of the elements of the eigenvector.

Simplest generalization of the method to more than 2 communities is to use repeated bisection
A simple model of modular networks

Model parameter $r$:
Ratio of inter- to intra-modular connection density

(a) $r = 0$

(b) $r = 0.1$

(c) $r = 1$

(d) Module $\equiv$ random network
Comparison with Watts-Strogatz model

Structural measures used:

Communication efficiency:
\[ E = [\text{avg path length}, \ell]^{-1} = \frac{2}{N(N-1)} \sum_{i>j} d_{ij} \]

Clustering coefficient:
\[ C = \frac{\text{fraction of observed to potential triads}}{\left(\frac{1}{N}\right) \sum_i 2n_i / k_i (k_i - 1)} \]

WS and Modular networks behave similarly as function of \( p \) or \( r \) (Also for between-ness centrality, edge clustering, etc)

In fact, for same \( N \) and \( \langle k \rangle \), we can find \( p \) and \( r \) such that the WS and Modular networks have the same “modularity” \( Q \)

Pan and Sinha, EPL (2009)
How can you tell them apart?

Dynamics on Modular networks different from that on Watts-Strogatz small-world networks

Consider synchronization on modular networks e.g., phase oscillators: \( \frac{d\theta_i}{dt} = w + \frac{1}{k_i} \sum K_{ij} \sin (\theta_j - \theta_i) \)

2 distinct time scales in Modular networks: \( t_{\text{modular}} \) & \( t_{\text{global}} \)
Existence of distinct time-scales in Modular networks

Consider linearized dynamics around synchronized state
\[ \frac{d\theta_i}{dt} = -\left(\frac{\kappa}{k_i}\right) \sum_j L_{ij} \theta_j, \quad (i = 1, \ldots, N) \]

Focus on the normal modes:
\[ \phi_i(t) = \sum_j B_{ij} \theta_j = \phi_i(0) \exp(-\lambda_i t), \quad (i = 1, \ldots, N) \]

- \( L \): Laplacian
- \( \kappa \): coupling strength of oscillators
- \( B \): matrix of eigenvectors
- \( \lambda_i \): eigenvalues
- \( D \): diagonal matrix s.t. \( D_{ii} = k_i \)

\[ L' = D^{-1/2} L = D^{1/2} L' D^{-1/2} \text{ is symmetric, normalized Laplacian} \implies \lambda_i \text{ real} \]

Differences in time-scales of modes \( \Rightarrow \) gap in spectrum of \( L \)

Mode for smallest \( \lambda_i \): associated with global synchronization
Other modes: synchronization within different groups of oscillators
Eigenvalue spectra of the Laplacian

Shows the existence of spectral gap $\Rightarrow$ distinct time scales

Modular network Laplacian spectra

Spectral gap in modular networks diverges with decreasing $r$

WS network Laplacian spectra

Existence of distinct time-scales in Modular networks

No such distinction in Watts-Strogatz small-world networks

Pan and Sinha, EPL (2009)
The networks of cortical connections in mammalian brain have been shown to have "small-world" structural properties.

Our analysis reveals their dynamical properties to be consistent with modular "small-world" networks.

Fast synchronization of neuronal activity within a module: The mechanism for efficient neural information processing?
How about other kinds of mesoscopic structures? E.g., Hierarchy

Hierarchical Modular networks

Modules may occur at different levels of hierarchy

Level 1: Modules A, B, C, D

Level 2: Meta-Modules I, II

- $r = 1$: randomly coupled network.
- $r = 0$: isolated sub-networks (modules)
- $0 < r < 1$: hierarchically structured network.
Hierarchical modularity in metabolic network

Topological overlap:

$$O_T(i, j) = \frac{\sum_{l=1}^{N} l_{i,l} \cdot l_{j,l} + l_{i,j}}{\min(k_i, k_j) + 1 - l_{i,j}}$$

Hierarchical Modular Networks exhibit several distinct time-scales — equal to the number of hierarchical levels (Sinha & Poria, 2011)

Synchronization of phase oscillators in hierarchical modular network show as many distinct time-scales as number of hierarchical levels ... Reflected in the eigenvalue spectra