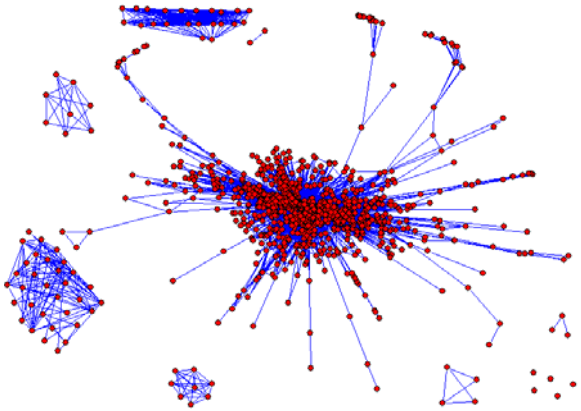


Network Construction
"A General Framework for Weighted Gene Co-Expression Network Analysis"

Steve Horvath
Human Genetics and Biostatistics
University of CA, LA

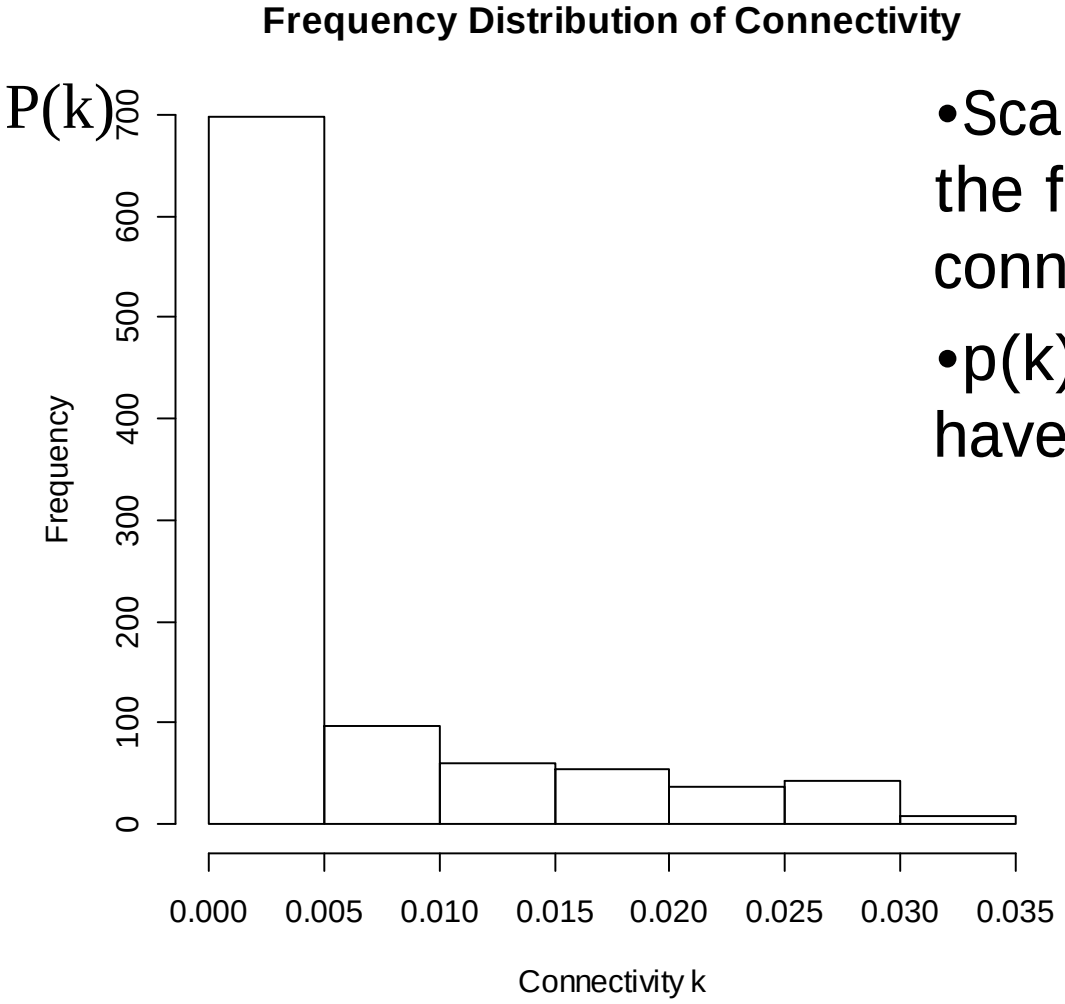
Background

- Network based methods have been found useful in many domains,
  - protein interaction networks
  - the world wide web
  - social interaction networks
  - OUR FOCUS: gene co-expression networks

Approximate scale free topology is a fundamental property of such networks (Barabasi et al)

- It entails the presence of hub nodes that are connected to a large number of other nodes
- Such networks are robust with respect to the random deletion of nodes but are sensitive to the targeted attack on hub nodes
- It has been demonstrated that metabolic networks exhibit scale free topology at least approximately.

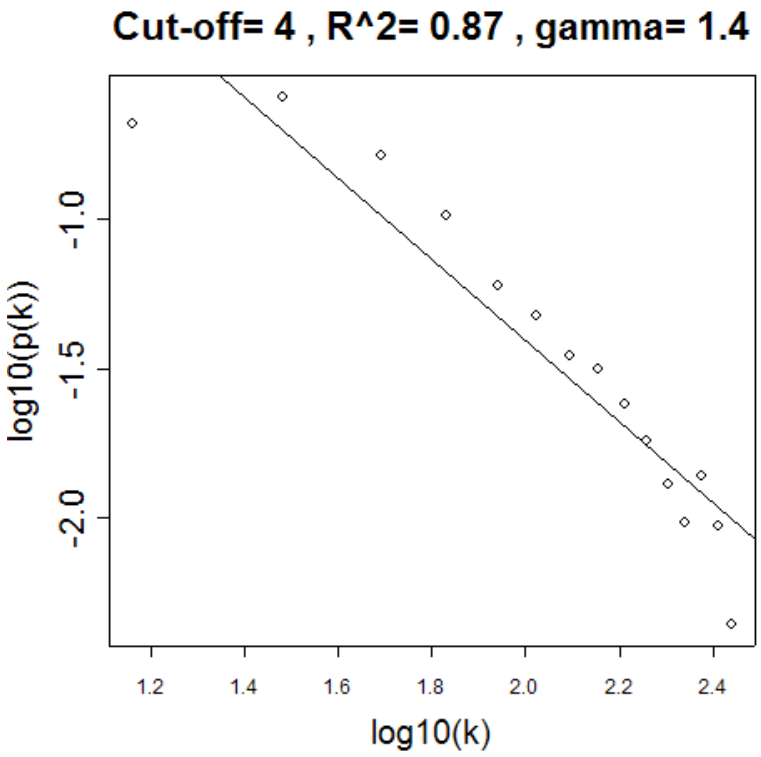
P(k) vs k in scale free networks



- Scale Free Topology refers to the frequency distribution of the connectivity k
- p(k)=proportion of nodes that have connectivity k

How to check Scale Free Topology?

Idea: Log transformation p(k) and k and look at scatter plots



Linear model fitting R^2 index can be used to quantify goodness of fit

Generalizing the notion of scale free topology

Motivation of generalizations: using weak general assumptions, we have proven that gene co-expression networks satisfy these distributions approximately.

Barabasi (1999)

$$ScaleFree \triangleq \log(p(k)) = c_0 + c_1 \log(k)$$

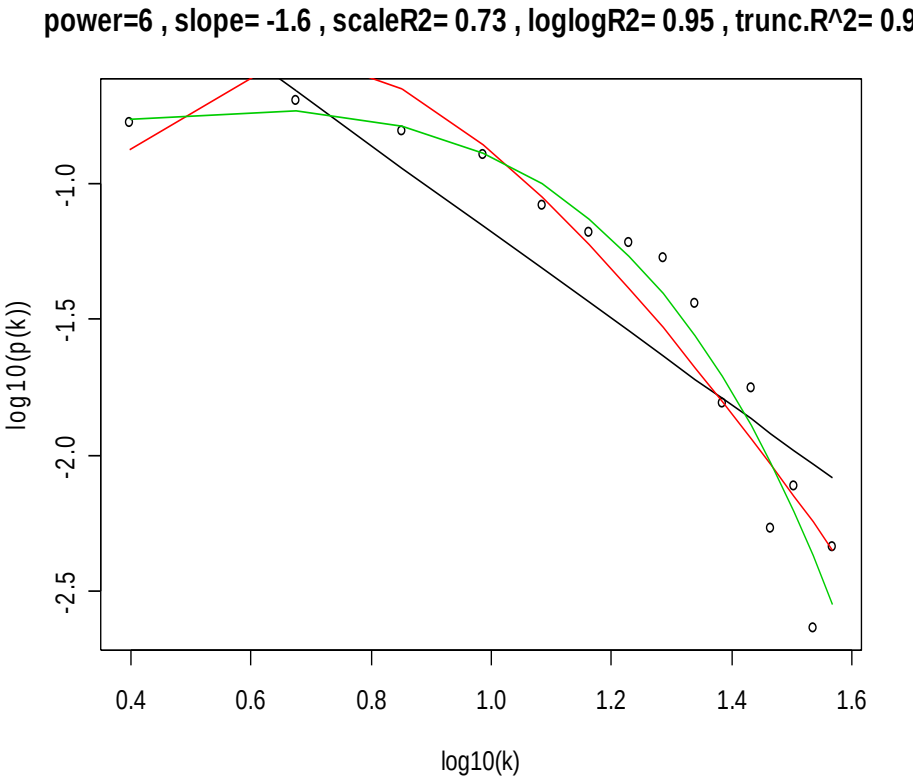
Csanyi-Szendroi (2004)

$$ExponentiallyTrunatedSFT \triangleq \log(p(k)) = c_0 + c_1 \log(k) + c_2 k$$

Horvath, Dong (2005)

$$LogLogSFT \triangleq \log(p(k)) = c_0 + c_1 \log(k) + c_2 \log(\log(k))$$

Checking Scale Free Topology in the Yeast Network

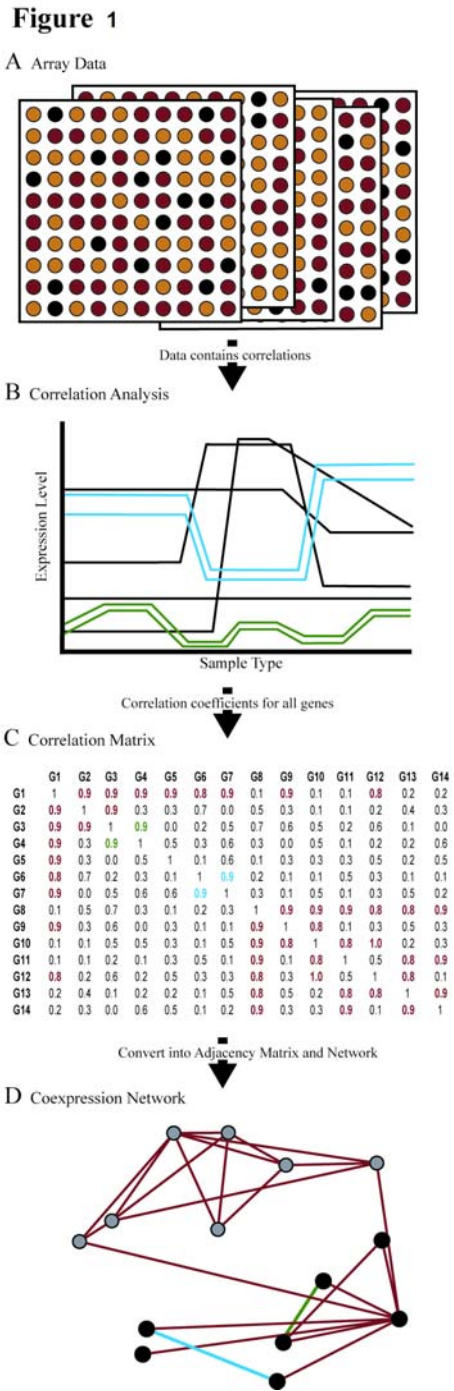


- Black=Scale Free
- Red=Exp. Truncated
- Green=Log Log SFT

How to define a gene co-expression network?

Gene Co-expression Networks

- In gene co-expression networks, each gene corresponds to a node.
- Two genes are connected by an edge if their expression values are highly correlated.
- Definition of “high” correlation is somewhat tricky
  - One can use statistical significance...
  - But we propose a criterion for picking threshold parameter: scale free topology criterion.



Steps for constructing a simple, unweighted co-expression network

A) Microarray gene expression data

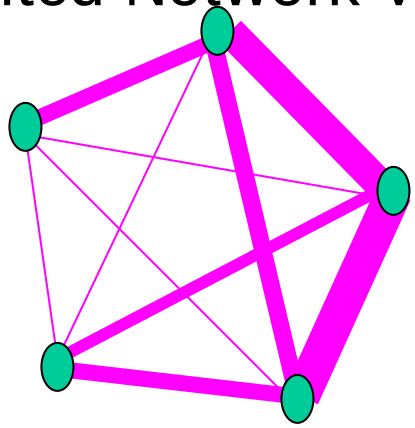
B) Measure concordance of gene expression with a Pearson correlation

C) The Pearson correlation matrix is dichotomized to arrive at an adjacency matrix. Binary values in the adjacency matrix correspond to an unweighted network.

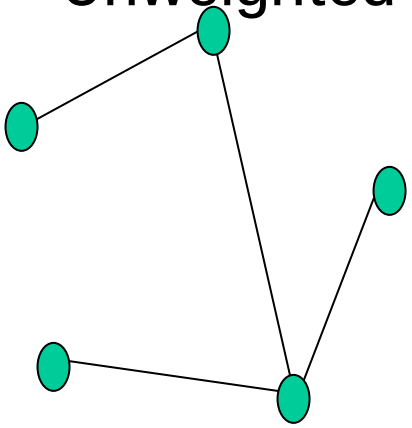
D) The adjacency matrix can be visualized by a graph.

Our `holistic' view....

Weighted Network View



Unweighted View



- All genes are connected
- Connection Widths=Connection strenghts

Some genes are connected
All connections are equal

Hard thresholding may lead to an information loss.

If two genes are correlated with r=0.79, they are deemed unconnected with regard to a hard threshold of tau=0.8

Mathematical Definition of an Undirected Network

Network=Adjacency Matrix

- A network can be represented by an adjacency matrix, $A=[a_{ij}]$, that encodes whether/how a pair of nodes is connected.
  - A is a symmetric matrix with entries in [0,1]
  - For unweighted network, entries are 1 or 0 depending on whether or not 2 nodes are adjacent (connected)
  - For weighted networks, the adjacency matrix reports the connection strength between gene pairs

Generalized Connectivity

- Gene connectivity = row sum of the adjacency matrix
  - For unweighted networks=number of direct neighbors
  - For weighted networks= sum of connection strengths to other nodes

$$k_i = \sum_j a_{ij}$$

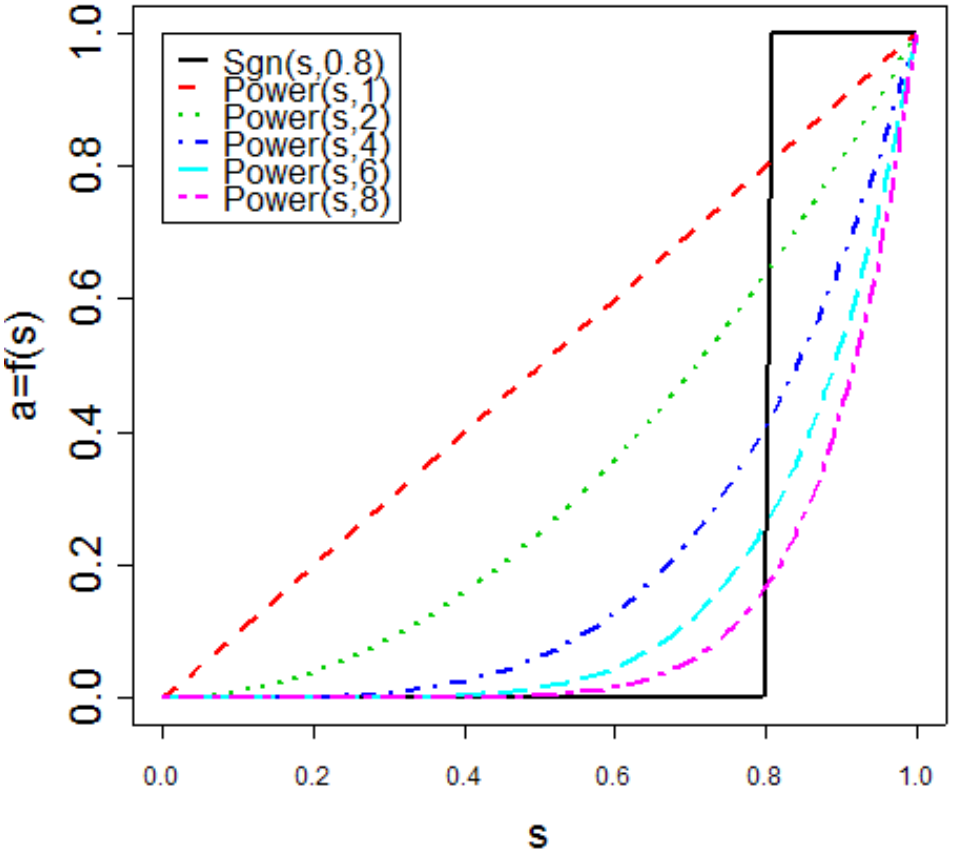
How to construct a weighted gene co-expression network?

Using an adjacency function to define a network

- Measure co-expression by a similarity s(i,j) in [0,1] e.g. absolute value of the Pearson correlation
- Define an adjacency matrix as A(i,j) using an adjacency function AF(s(i,j))
- Abstractly speaking an adjacency function AF is a monotonic function from [0,1] onto [0,1]
- Here we consider 2 classes of AFs
  - Step function AF(s)=I(s>tau) with parameter tau (unweighted network)
  - Power function $AF(s)=s^b$ with parameter b
- The choice of the AF parameters (tau, b) determines the properties of the network.

Comparing the power adjacency functions with the step function

Adjacency
=connection strength



Gene Co-expression Similarity

The scale free topology criterion for choosing the parameter values of an adjacency function.

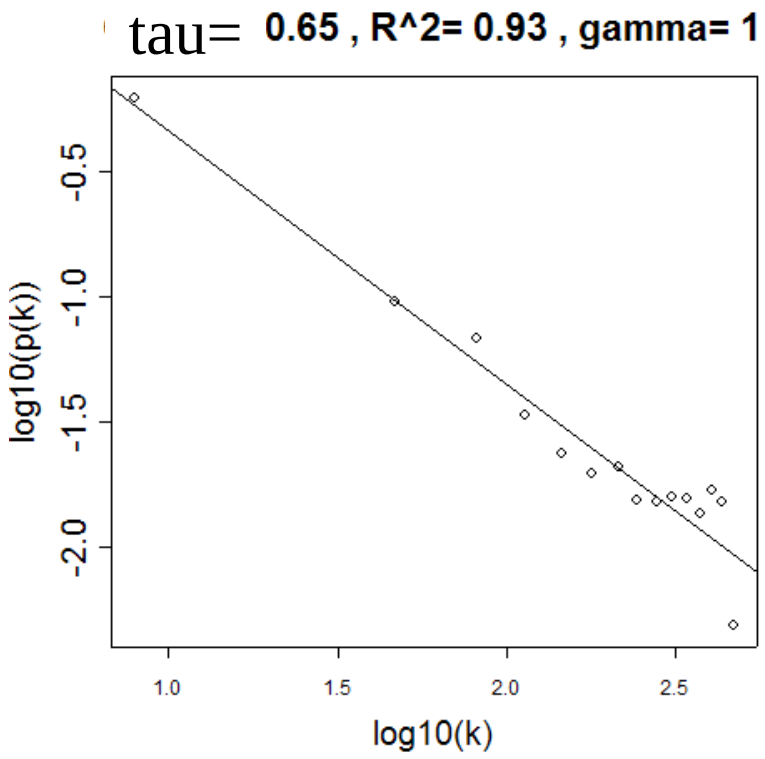
A) CONSIDER ONLY THOSE PARAMETER VALUES THAT RESULT IN *APPROXIMATE* SCALE FREE TOPOLOGY

B) SELECT THE PARAMETERS THAT RESULT IN THE HIGHEST MEAN NUMBER OF CONNECTIONS

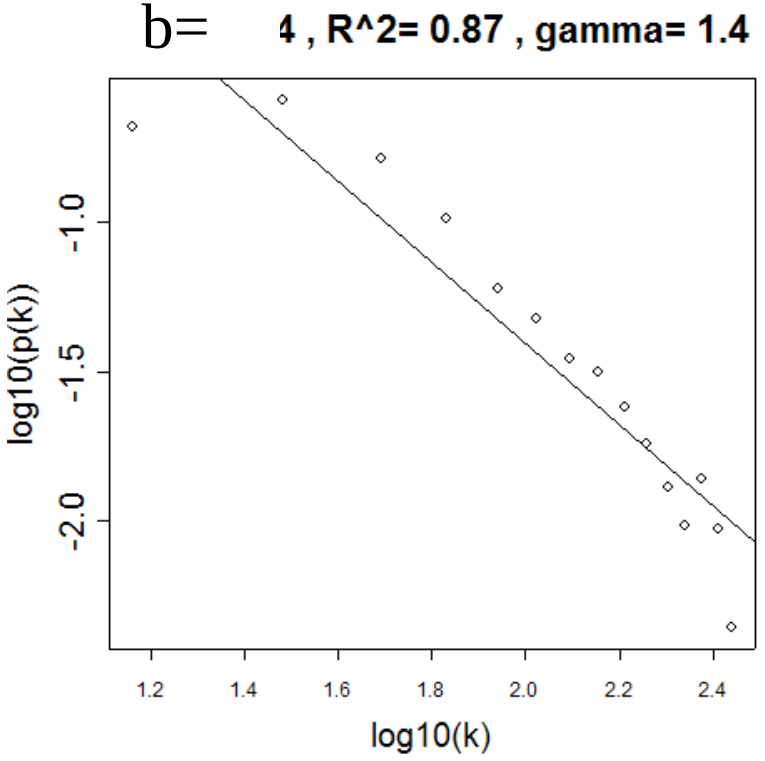
- Criterion A is motivated by the finding that most metabolic networks (including gene co-expression networks, protein-protein interaction networks and cellular networks) have been found to exhibit a scale free topology
- Criterion B leads to high power for detecting modules (clusters of genes) and hub genes.

Criterion A is measured by the linear model fitting index R^2

Step AF (tau)



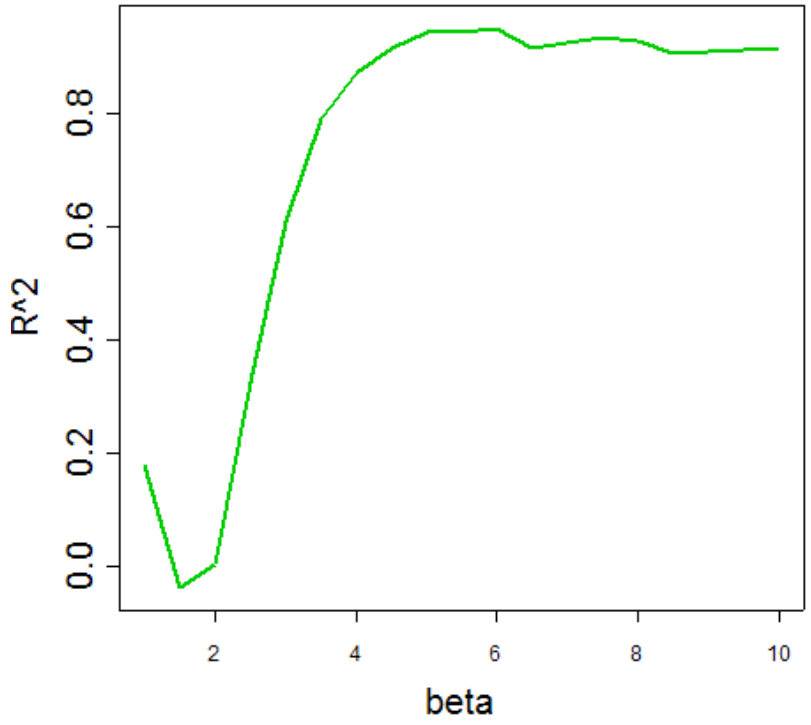
Power AF (b)



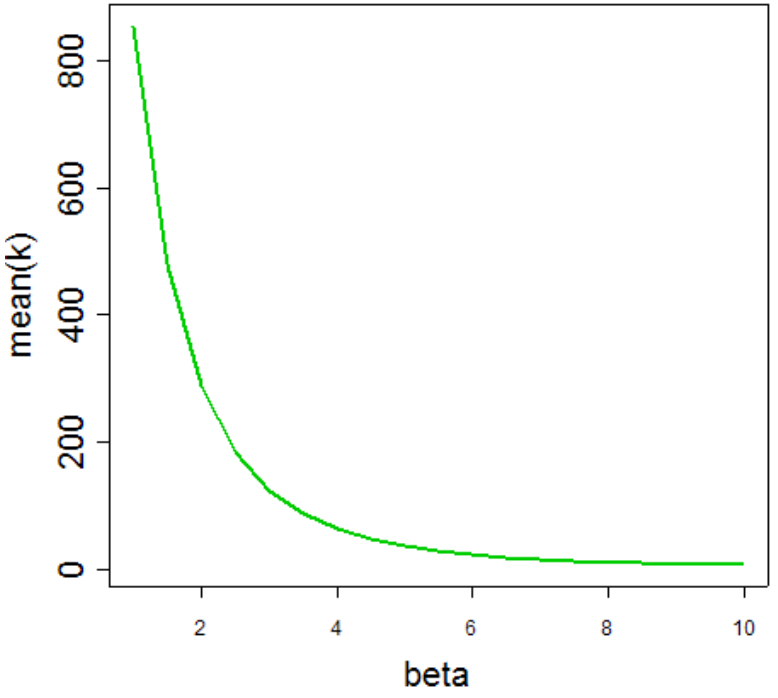
Trade-off between criterion A (R^2) and criterion B (mean no. of connections) when varying the power b

Power AF(s)=s^b

criterion A: SFT model fit R^2



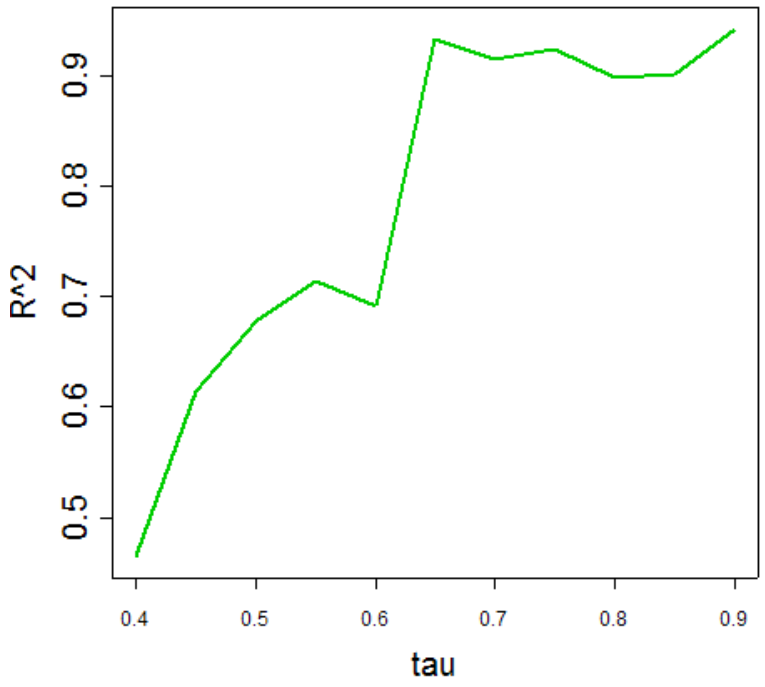
criterion B: mean connectivity



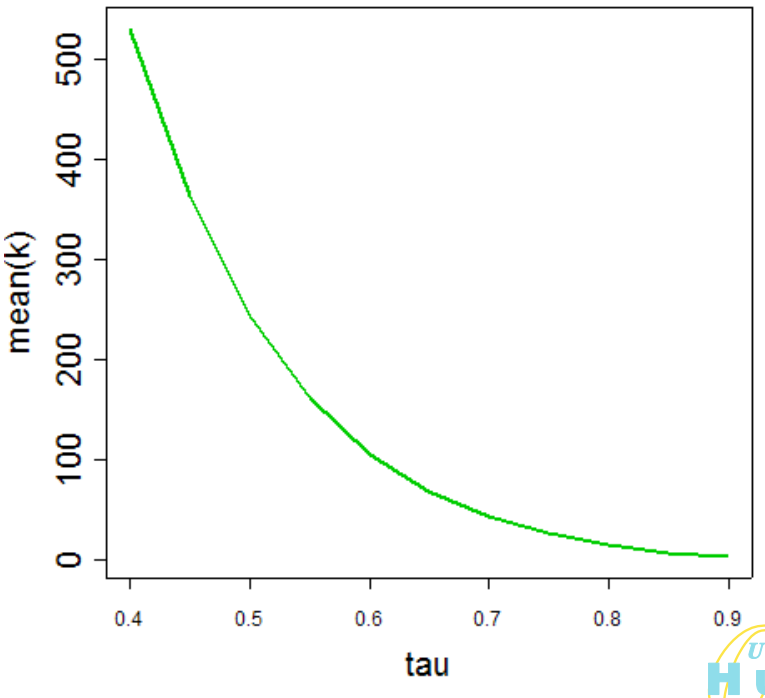
Trade-off between criterion A and B when varying tau

Step Function: I(s>tau)

criterion A

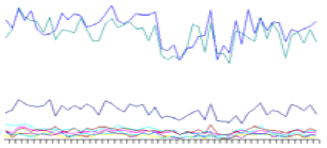


criterion B

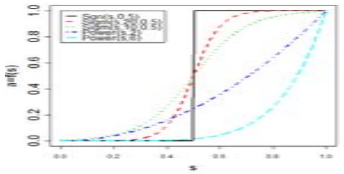


General Framework for Network Analysis

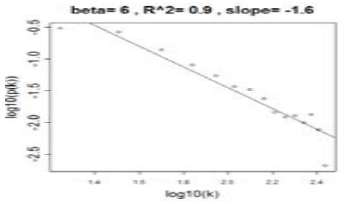
Define a Gene Co-expression Similarity



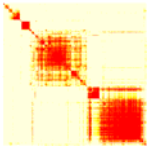
Define a Family of Adjacency Functions



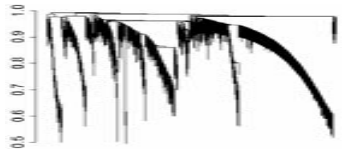
Determine the AF Parameters



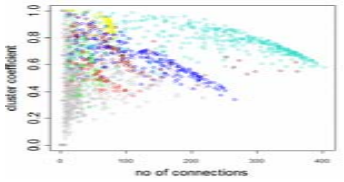
Define a Measure of Node Dissimilarity



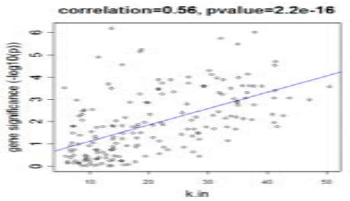
Identify Network Modules (Clustering)



Relate Network Concepts to Each Other



Relate the Network Concepts to External Gene or Sample Information



How to measure distance in a network?

- Mathematical Answer: Geodesics
  - length of shortest path connecting 2 nodes
- Biological Answer: look at shared neighbors
  - Intuition: if 2 people share the same friends they are close in a social network
  - Use the topological overlap measure based distance proposed by Ravasz et al (2002)

Topological Overlap leads to a network distance measure (Ravasz et al 2002)

$$TOM_{ij} = \frac{\sum_u a_{iu} a_{uj} + a_{ij}}{\min(k_i, k_j) + 1 - a_{ij}}$$

$$DistTOM_{ij} = 1 - TOM_{ij}$$

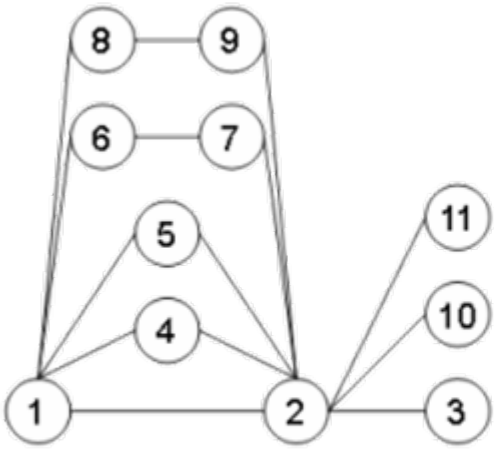
- Generalized in Zhang and Horvath (2005) to the case of weighted networks.

Set theoretic interpretation of the topological overlap measure. Empirical studies of its robustness.

- Yip A, Horvath S (2007) Gene network interconnectedness and the generalized topological overlap measure. BMC Bioinformatics 20078:22
- Li A, Horvath S (2006) Network Neighborhood Analysis with the multi-node topological overlap measure. Bioinformatics. doi:10.1093/bioinformatics/btl581

The general topological overlap matrix

a.



b.

$N_m(i)$	$i=1$	$i=2$	$i=3$
$m=1$	2,4,5,6,8	1,3,4,5,7,9,10,11	2
$m=2$	2,3,4,5,6,7,8,9,10,11	1,3,4,5,6,7,8,9,10,11	1,2,4,5,7,9,10,11

c.

$t_{ij}^{[m]}$	$(i,j)=(1,2)$	$(i,j)=(1,3)$	$(i,j)=(2,3)$
$m=0$	1	0	1
$m=1$	3/5	1/2	1
$m=2$	1	7/9	1

$$TOM(i,j)=\frac{|N_1(i)\cap N_1(j)|+a_{ij}}{\min(|N_1(i)|,|N_1(j)|)+1-a_{ij}}$$

$N_1(i)$ denotes the set of neighbors of node i

|*| measures the cardinality

Yip, Horvath (2005)

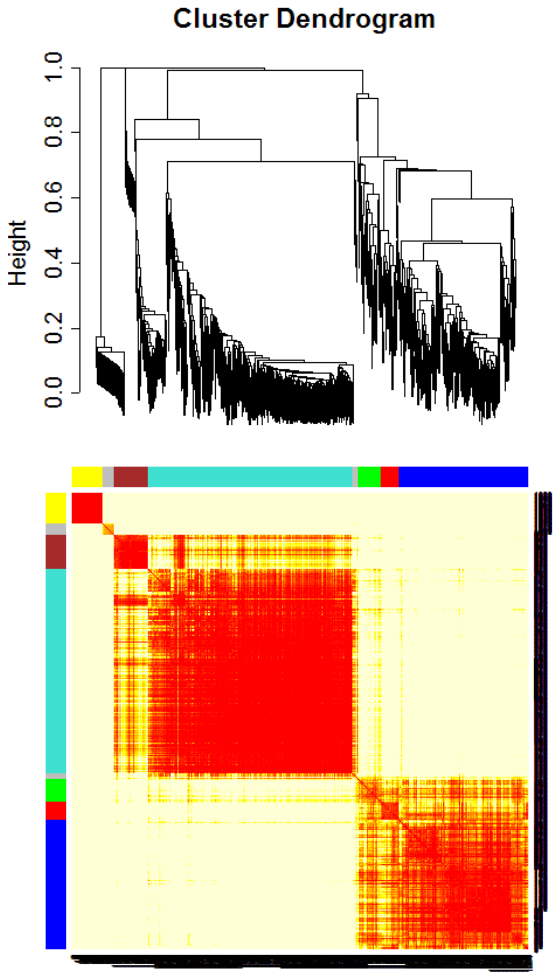
Defining Gene Modules
=sets of tightly co-regulated genes

Module Identification based on the notion of topological overlap

- One important aim of metabolic network analysis is to detect subsets of nodes (modules) that are tightly connected to each other.
- We adopt the definition of Ravasz et al (2002): modules are groups of nodes that have high topological overlap.

Steps for defining gene modules

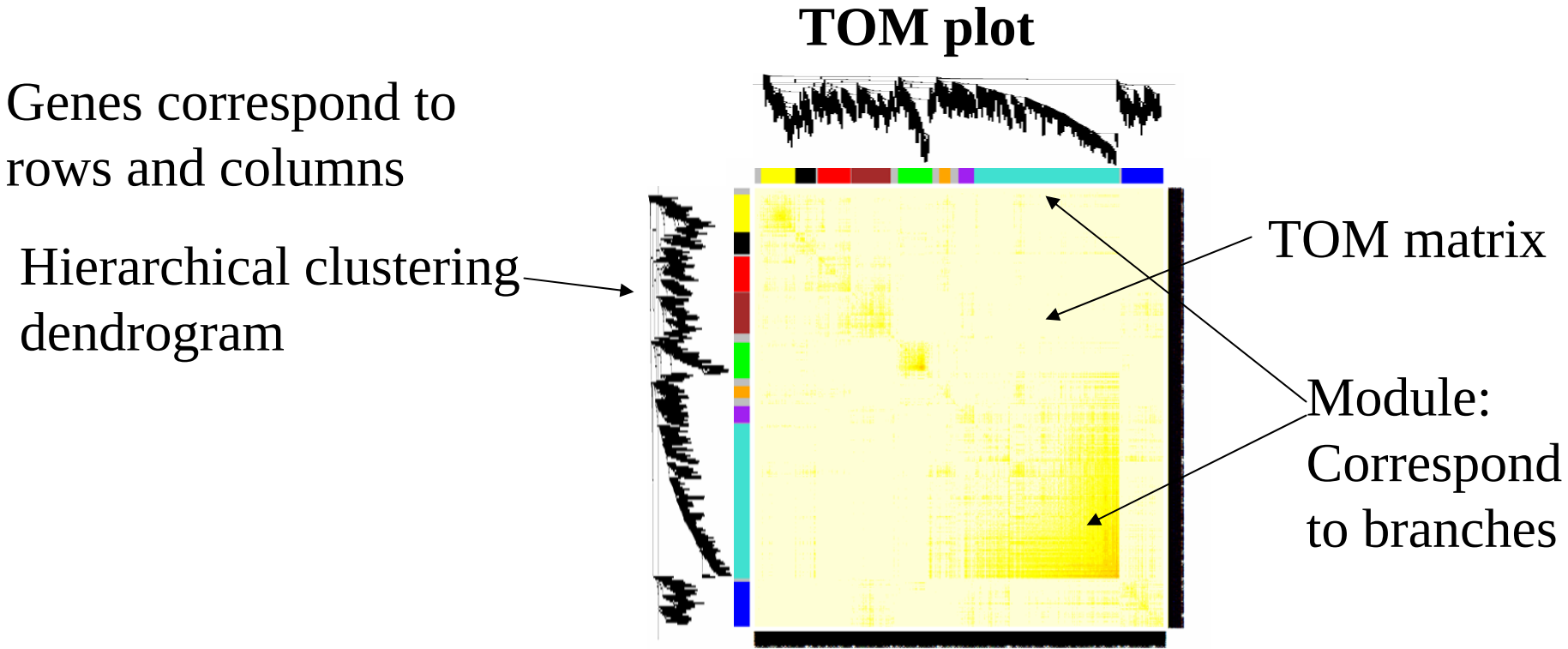
- Define a dissimilarity measure between the genes.
  - Standard Choice: dissim(i,j)=1-abs(correlation)
  - Choice by network community=1-Topological Overlap Matrix (TOM)
    - Used here
- Use the dissimilarity in hierarchical clustering
- Define modules as branches of the hierarchical clustering tree
- Visualize the modules and the clustering results in a heatmap plot



Heatmap

Using the TOM matrix to cluster genes

- To group nodes with high topological overlap into modules (clusters), we typically use average linkage hierarchical clustering coupled with the TOM distance measure.
- Once a dendrogram is obtained from a hierarchical clustering method, we choose a height cutoff to arrive at a clustering.
  - Here modules correspond to branches of the dendrogram

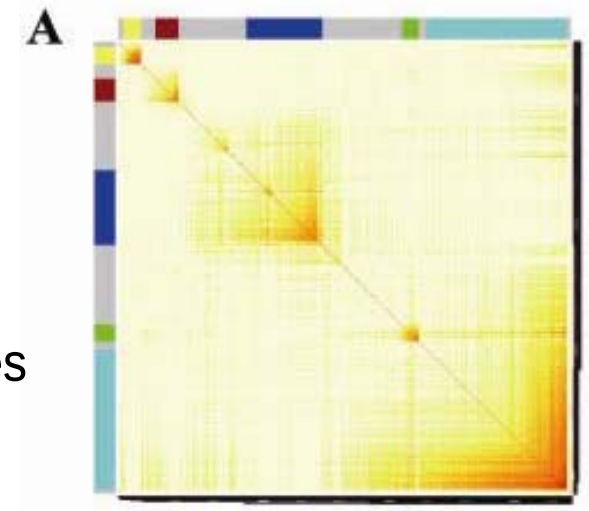


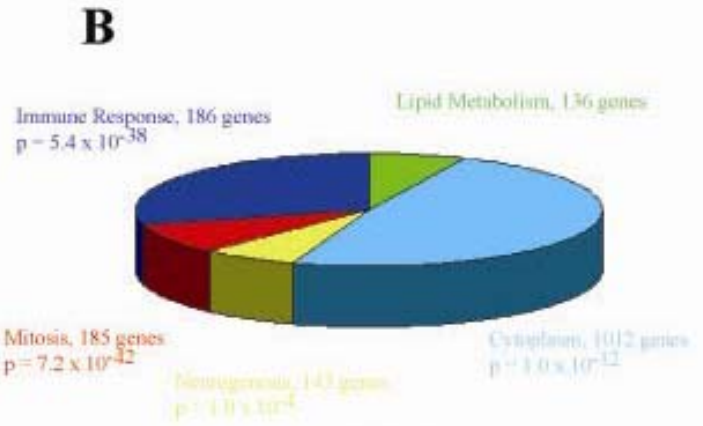
Different Ways of Depicting Gene Modules

Topological Overlap Plot

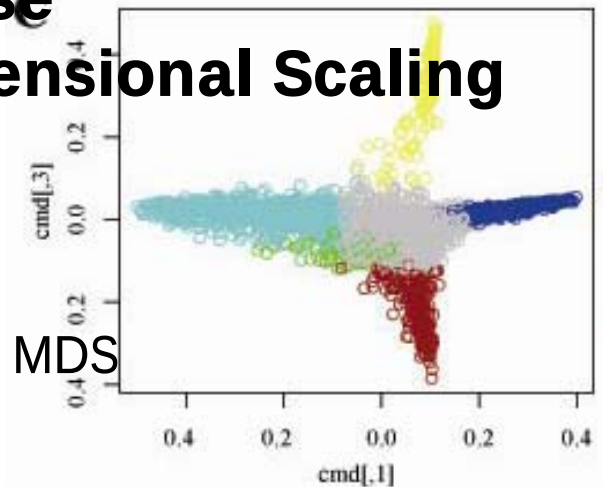
Gene Functions

1) Rows and columns correspond to genes
2) Red boxes along diagonal are modules
3) Color bands=modules

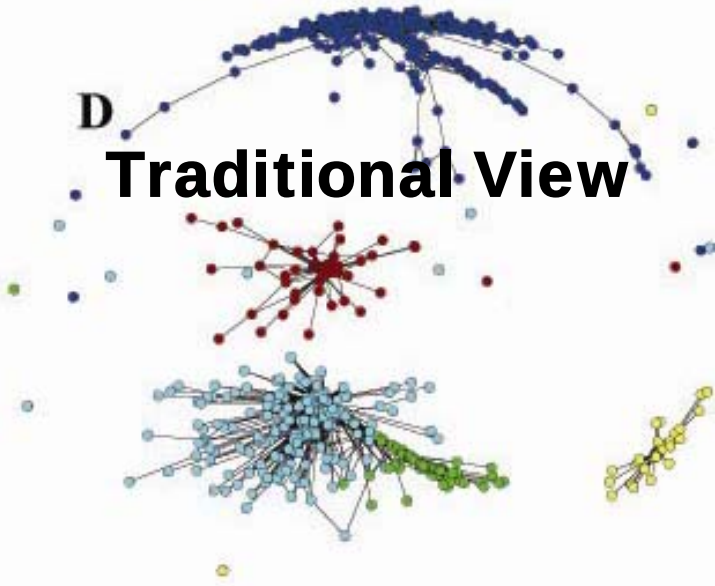




We propose Multi Dimensional Scaling



Traditional View

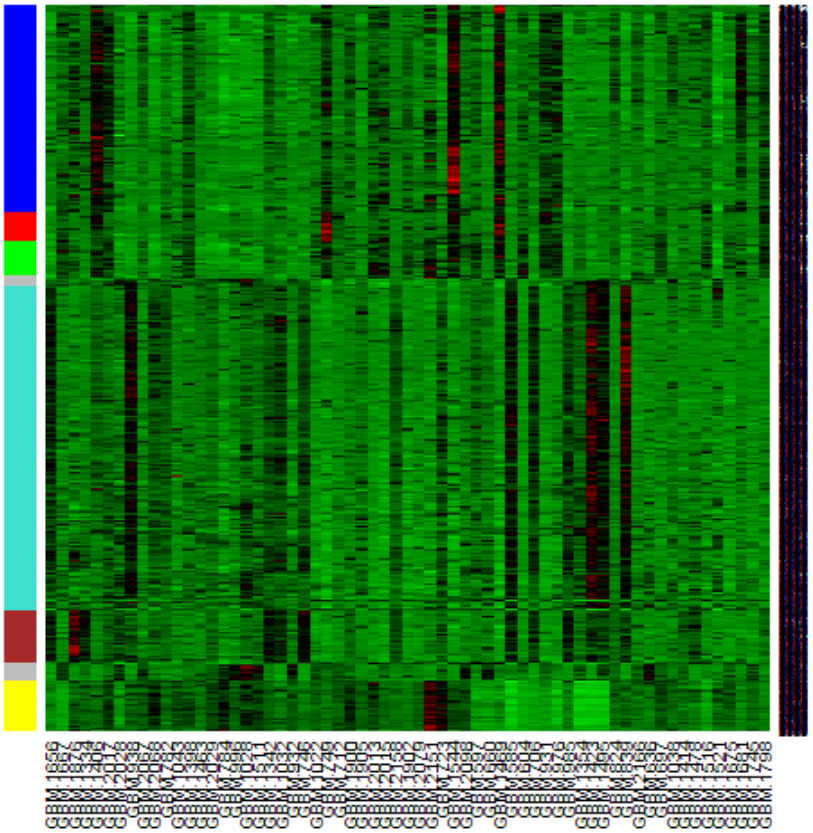


Idea:
Use network distance in MDS

More traditional view of module

Columns=Brain tissue samples

Rows=Genes
Color band indicates module membership

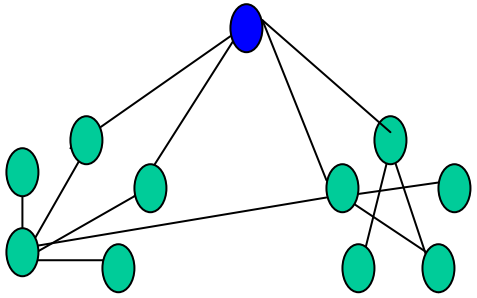


Message: characteristic vertical bands indicate tight co-expression of module genes

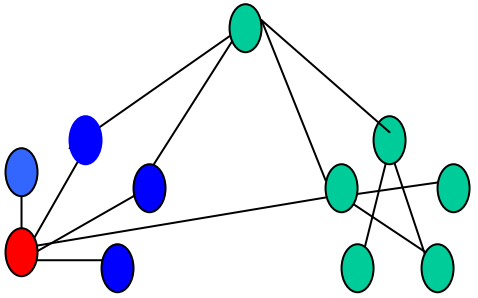
Module-Centric View of Networks

Module-centric view (intramodular connectivity) v.s. whole network view (whole network connectivity)

- Traditional view based on whole network connectivity



- Module view based on within module connectivity



In many applications, we find that intramodular connectivity is biologically and mathematically more meaningful than whole network connectivity

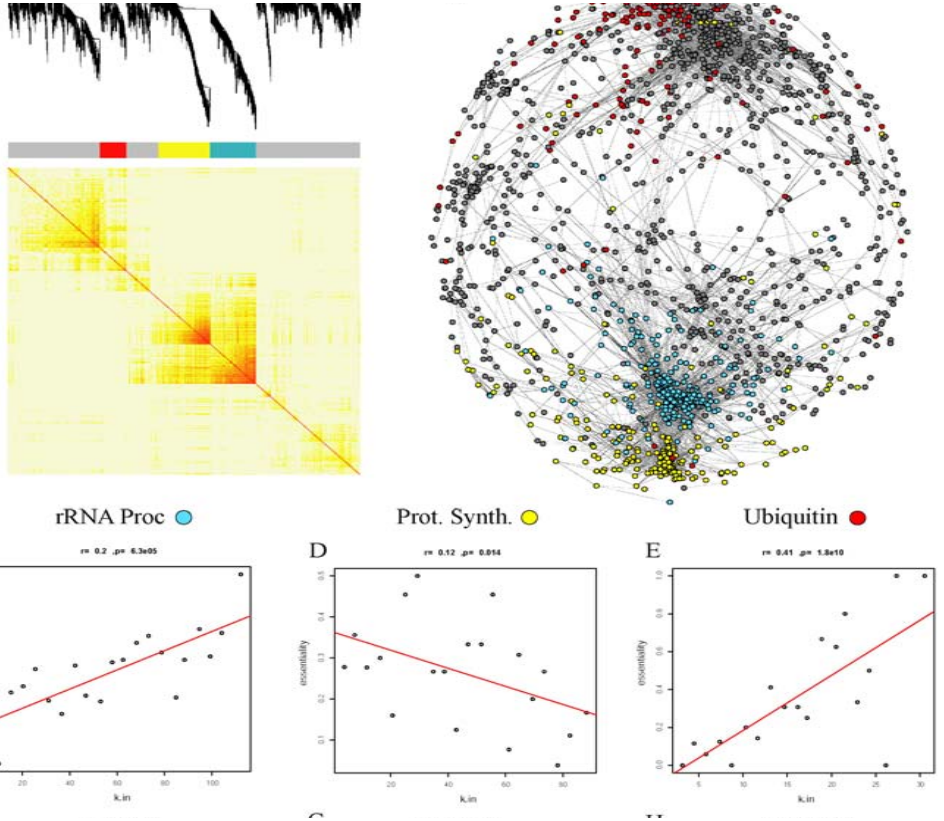
Mathematical Facts in our gene co-expression networks
Hub genes are always module genes in co-expression networks.
Most module genes have high connectivity.

Yeast Data Analysis
Marc Carlson

Within Module Analysis

Findings

1) The intramodular connectivities are related to how essential a gene is for yeast survival
2) Modules are highly preserved across different data sets
3) Hub genes are highly preserved across species



Prob(Essential)

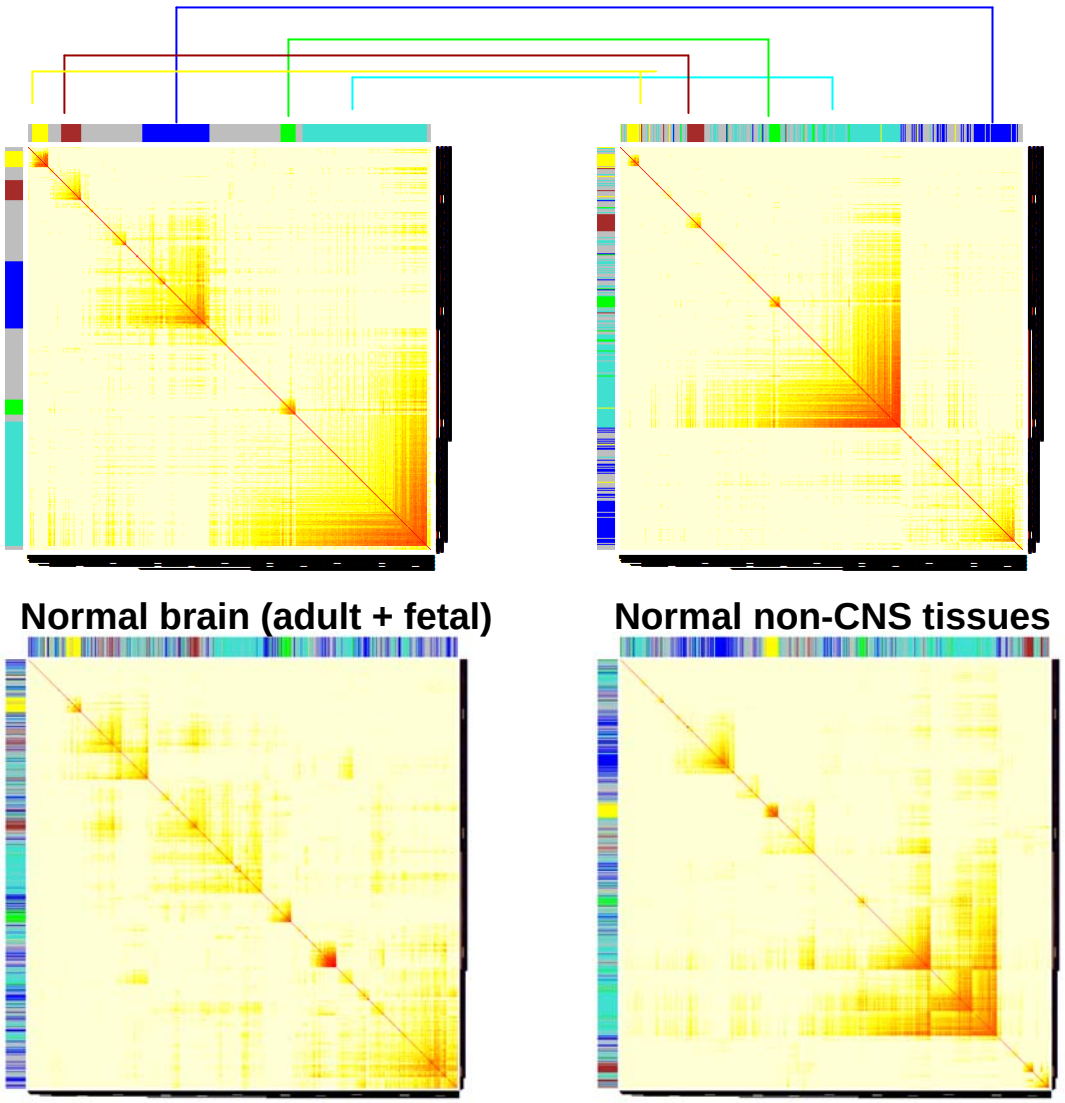
Connectivity k

Details: "Gene Connectivity, Function, and Sequence Conservation: Predictions from Modular Yeast Co-Expression Networks" (2006) by Carlson MRJ, Zhang B, Fang Z, Mischel PS, Horvath S, and Nelson SF, BMC Genomics 2006, 7:40

Intramodular hub genes in a relevant module predict brain cancer survival.

Horvath S, Zhang B, Carlson M, Lu KV, Zhu S, Felciano RM, Laurance MF, Zhao W, Shu, Q, Lee Y, Scheck AC, Liau LM, Wu H, Geschwind DH, Febbo PG, Kornblum HI, Cloughesy TF, Nelson SF, Mischel PS (2006) "Analysis of Oncogenic Signaling Networks in Glioblastoma Identifies ASPM as a Novel Molecular Target", PNAS | November 14, 2006 | vol. 103 | no. 46 | 17402-17407

Module structure is highly preserved across data sets



Messages:
1) Cancer modules can be independently validated
2) Modules in brain cancer tissue can also be found in normal, non-brain tissue.
-->
Insights into the biology of cancer

Gene prognostic significance

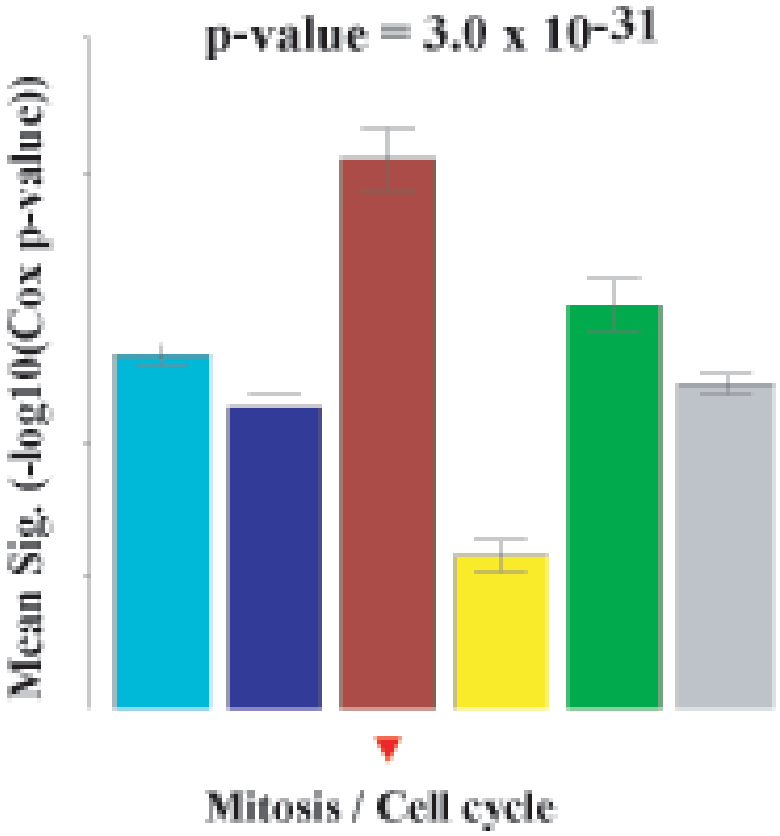
Definition

1) Regress survival time on gene expression information using a univariable Cox regression model
2) Obtain the score test p-value
3) Gene significance=-log10(p-value)
   - Roughly speaking
     Gene significance~no of zeroes in the p-value.

Goal

Relate gene significance to intramodular connectivity

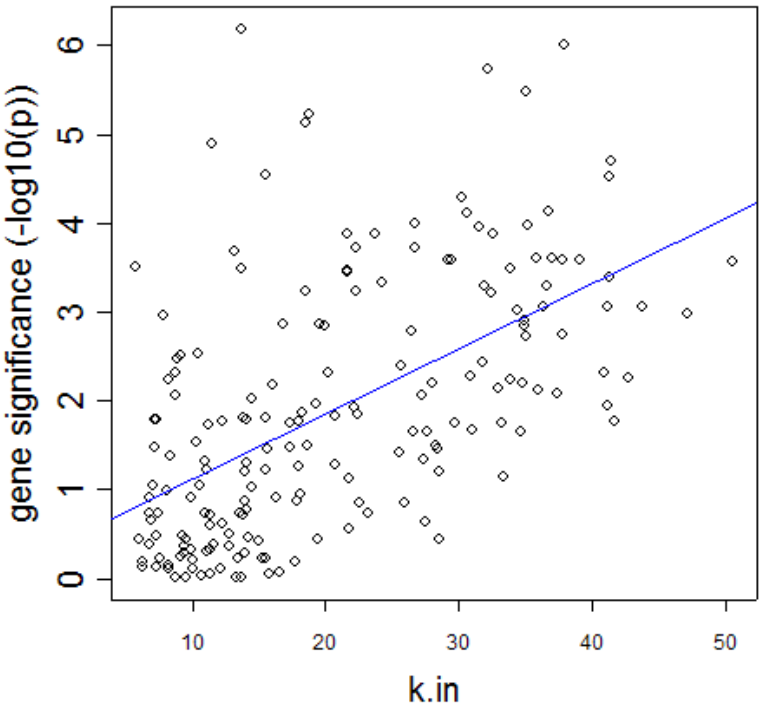
Mean Prognostic Significance of Module Genes



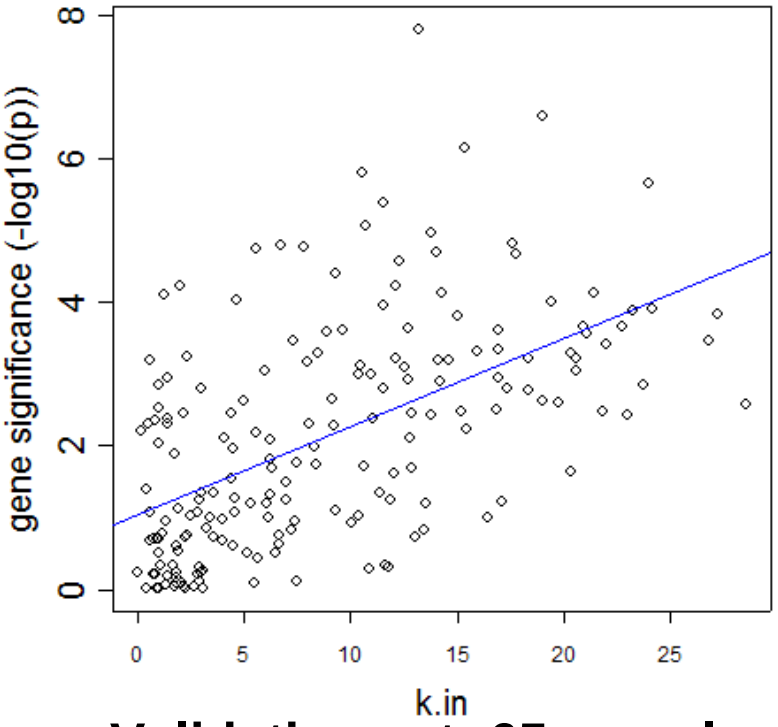
Message: Focus the attention on the brown module genes

Module hub genes predict cancer survival

1. **Intramodular connectivity is highly correlated with gene significance**
2. **Recall prognostic significance as –log10(Cox-p-value)**



Test set: 55 samples
r = 0.56; p-2.2 x 10^{-16}



Validation set: 65 samples
r = 0.55; p-2.2 x 10^{-16}

The fact that genes with high intramodular connectivity are more likely to be prognostically significant facilitates a novel screening strategy for finding prognostic genes

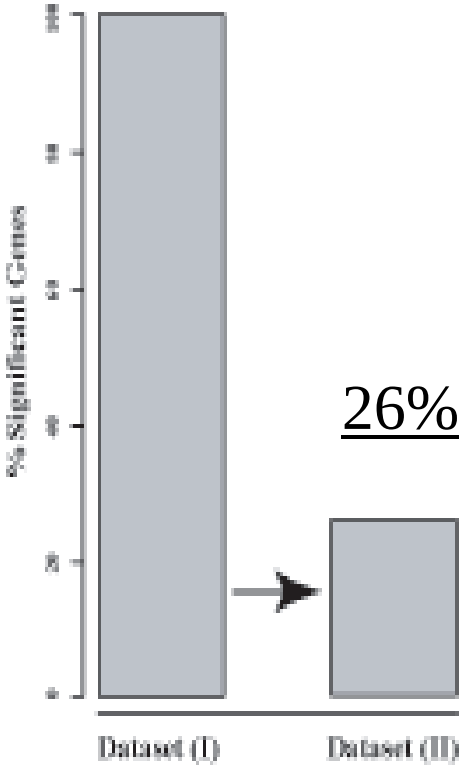
- Focus on those genes with significant Cox regression p-value and high intramodular connectivity.
  - It is essential to to take a module centric view: focus on intramodular connectivity of module that is enriched with significant genes.

Gene screening strategy that makes use of intramodular connectivity is far superior to standard approach

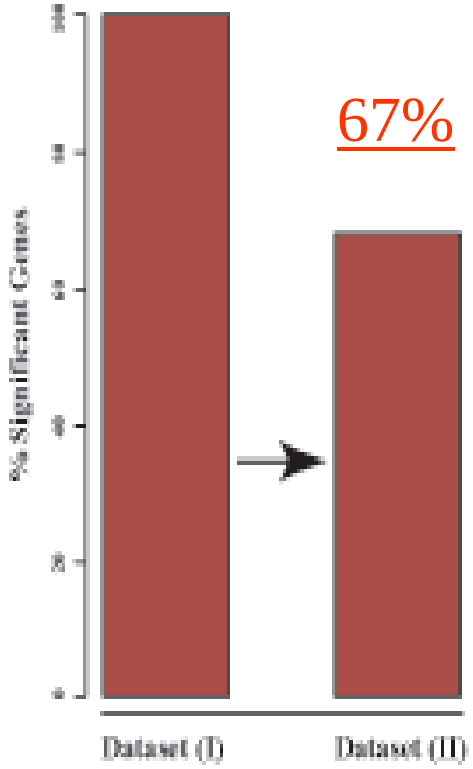
- Validation success rate= proportion of genes with independent test set Cox regression p-value<0.05.
- Validation success rate of network based screening approach (68%)
- Standard approach involving top 300 most significant genes: 26%

Validation success rate of gene expressions in independent data

300 most significant genes
(Cox p-value<$1.3*10^{-3}$)



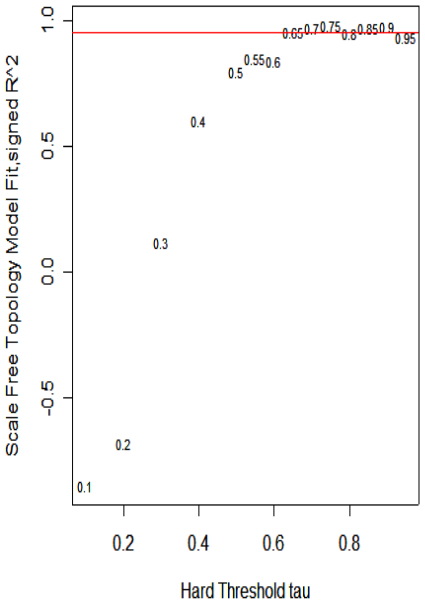
Network based screening
p<0.05 and
high intramodular connectivity

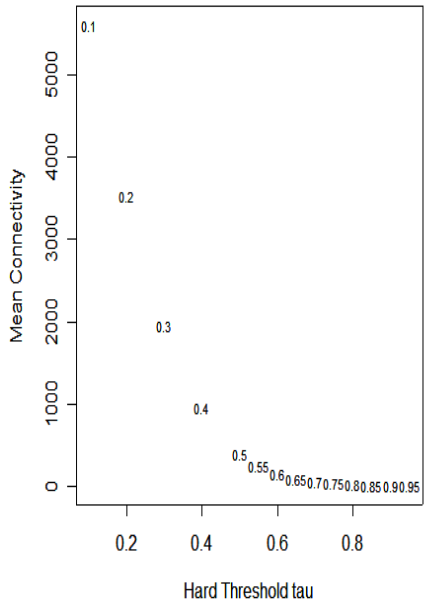


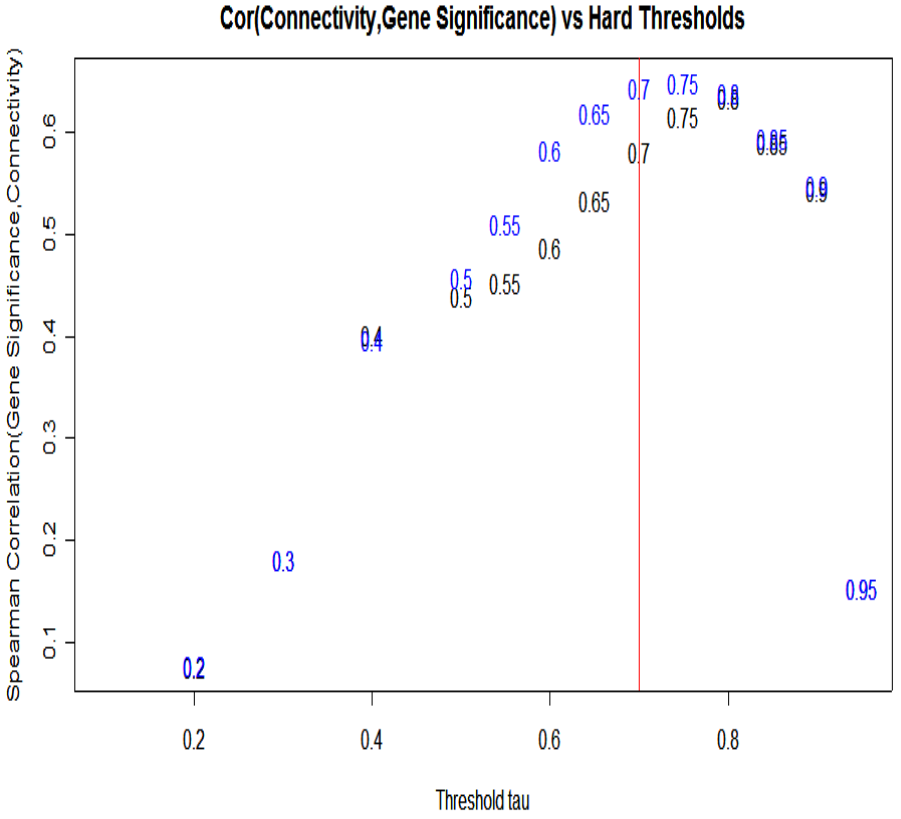
The biological signal is much more robust in weighted than in unweighted networks.

- Biological signal = Spearman correlation between brown intramodular connectivity and prognostic significance,
  - Biological Signal=cor(Gene Signif ,K)
- Robustness analysis
  - Explore how this biological signal changes as a function of the adjacency function parameters tau (hard thresholding) and b (=power=soft thresholding).

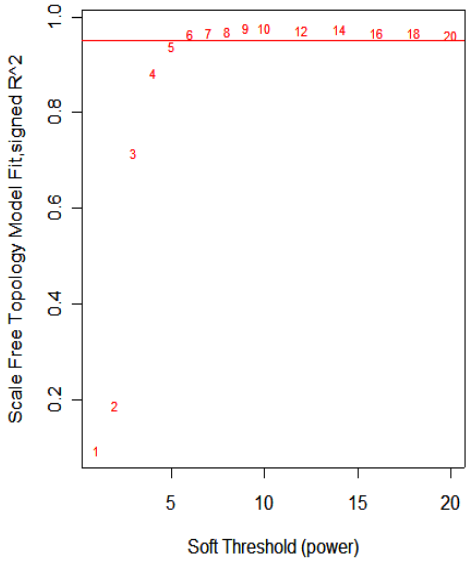
Scale Free Topology fitting index and biological signals for different hard thresholds

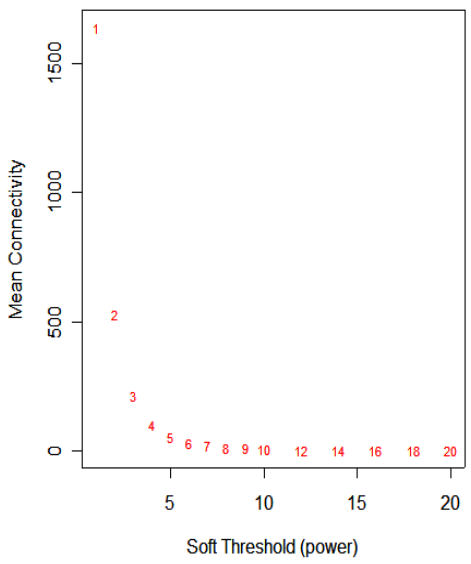


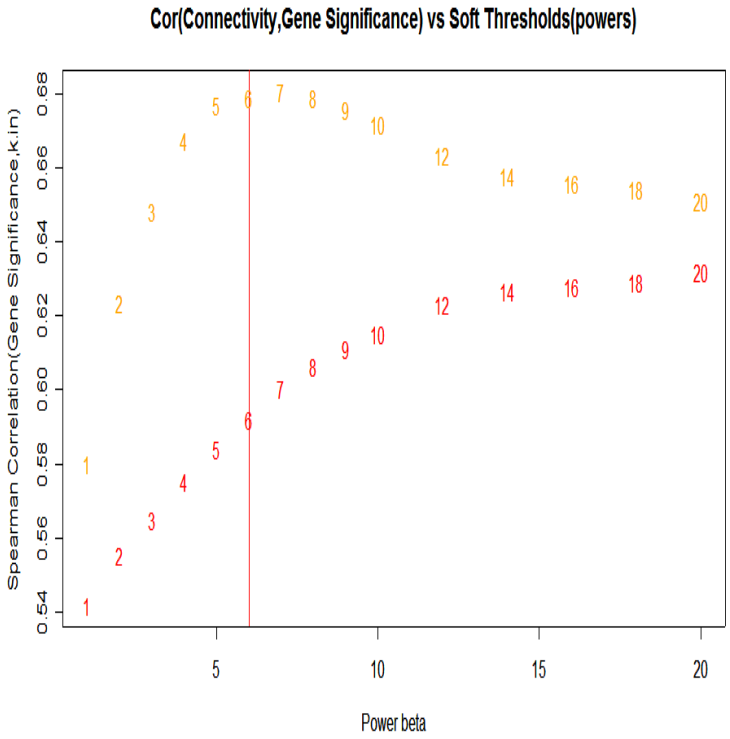




Scale Free Topology fitting index and biological signals for different SOFT thresholds (powers)







Soft thresholding leads to more robust results

- The results of soft thresholding are highly robust with respect to the choice of the adjacency function parameter, i.e. the power b
- In contrast, the results of hard thresholding are sensitive to the choice of tau
- In this application, the biological signal peaks close to the adjacency function parameter that was chosen by the scale free topology criterion.

Conclusion

- Gene co-expression network analysis can be interpreted as the study of the Pearson correlation matrix.
- Key insight: connectivity can be used to single out important genes.
- Weak relationship with principal or independent component analysis
  - Network methods focus on “local” properties
- Open questions:
  - What is the mathematical meaning of the scale free topology criterion
    - Starting point: noise suppression in modules.
  - Alternative connectivity measures, network distance measures
  - Which and how many genes to target to disrupt a disease module?

Main reference for this talk

•*Bin Zhang and Steve Horvath (2005) "A General Framework for Weighted Gene Co-Expression Network Analysis", Statistical Applications in Genetics and Molecular Biology: Vol. 4: No. 1, Article 17.*
http://www.bepress.com/sagmb/vol4/iss1/art17

•R software tutorials at

http://www.genetics.ucla.edu/labs/horvath/CoexpressionNetwork/

Google search "co-expression network"

A short methodological summary of the publications.

- How to construct a gene co-expression network using the scale free topology criterion? Robustness of network results. Relating a gene significance measure and the clustering coefficient to intramodular connectivity:
  - Zhang B, Horvath S (2005) "A General Framework for Weighted Gene Co-Expression Network Analysis", Statistical Applications in Genetics and Molecular Biology: Vol. 4: No. 1, Article 17
- Theory of module networks (both co-expression and protein-protein interaction modules):
  - Dong J, Horvath S (2007) Understanding Network Concepts in Modules, BMC Systems Biology 2007, 1:24
- What is the topological overlap measure? Empirical studies of the robustness of the topological overlap measure:
  - Yip A, Horvath S (2007) Gene network interconnectedness and the generalized topological overlap measure. BMC Bioinformatics 2007, 8:22
- Software for carrying out neighborhood analysis based on topological overlap. The paper shows that an initial seed neighborhood comprised of 2 or more highly interconnected genes (high TOM, high connectivity) yields superior results. It also shows that topological overlap is superior to correlation when dealing with expression data.
  - Li A, Horvath S (2006) Network Neighborhood Analysis with the multi-node topological overlap measure. Bioinformatics. doi:10.1093/bioinformatics/btl581
- Gene screening based on intramodular connectivity identifies brain cancer genes that validate. This paper shows that WGCNA greatly alleviates the multiple comparison problem and leads to reproducible findings.
  - Horvath S, Zhang B, Carlson M, Lu KV, Zhu S, Felciano RM, Laurance MF, Zhao W, Shu, Q, Lee Y, Scheck AC, Liau LM, Wu H, Geschwind DH, Febbo PG, Kornblum HI, Cloughesy TF, Nelson SF, Mischel PS (2006) "Analysis of Oncogenic Signaling Networks in Glioblastoma Identifies ASPM as a Novel Molecular Target", PNAS | November 14, 2006 | vol. 103 | no. 46 | 17402-17407
- The relationship between connectivity and knock-out essentiality is dependent on the module under consideration. Hub genes in some modules may be non-essential. This study shows that intramodular connectivity is much more meaningful than whole network connectivity:
  - "Gene Connectivity, Function, and Sequence Conservation: Predictions from Modular Yeast Co-Expression Networks" (2006) by Carlson MRJ, Zhang B, Fang Z, Mischel PS, Horvath S, and Nelson SF, BMC Genomics 2006, 7:40
- How to integrate SNP markers into weighted gene co-expression network analysis? The following 2 papers outline how SNP markers and co-expression networks can be used to screen for gene expressions underlying a complex trait. They also illustrate the use of the module eigengene based connectivity measure kME.
  - Single network analysis: Ghazalpour A, Doss S, Zhang B, Wang S, Plaisier C, Castellanos R, Brozell A, Schadt EE, Drake TA, Lusis AJ, Horvath S (2006) "Integrating Genetic and Network Analysis to Characterize Genes Related to Mouse Weight". PLoS Genetics. Volume 2 | Issue 8 | AUGUST 2006
  - Differential network analysis: Fuller TF, Ghazalpour A, Aten JE, Drake TA, Lusis AJ, Horvath S (2007) "Weighted Gene Co-expression Network Analysis Strategies Applied to Mouse Weight", Mammalian Genome. In Press
- The following application presents a `supervised' gene co-expression network analysis. In general, we prefer to construct a co-expression network and associated modules without regard to an external microarray sample trait (unsupervised WGCNA). But if thousands of genes are differentially expressed, one can construct a network on the basis of differentially expressed genes (supervised WGCNA):
  - Gargalovic PS, Imura M, Zhang B, Gharavi NM, Clark MJ, Pagnon J, Yang W, He A, Truong A, Patel S, Nelson SF, Horvath S, Berliner J, Kirchgessner T, Lusis AJ (2006) Identification of Inflammatory Gene Modules based on Variations of Human Endothelial Cell Responses to Oxidized Lipids. PNAS 22;103(34):12741-6
- The following paper presents a differential co-expression network analysis. It studies module preservation between two networks. By screening for genes with differential topological overlap, we identify biologically interesting genes. The paper also shows the value of summarizing a module by its module eigengene.
  - Oldham M, Horvath S, Geschwind D (2006) Conservation and Evolution of Gene Co-expression Networks in Human and Chimpanzee Brains. 2006 Nov 21;103(47):17973-8

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