Biological Network Analysis

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Yale

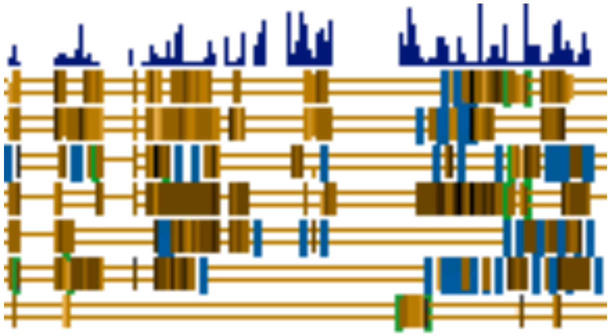
slides at

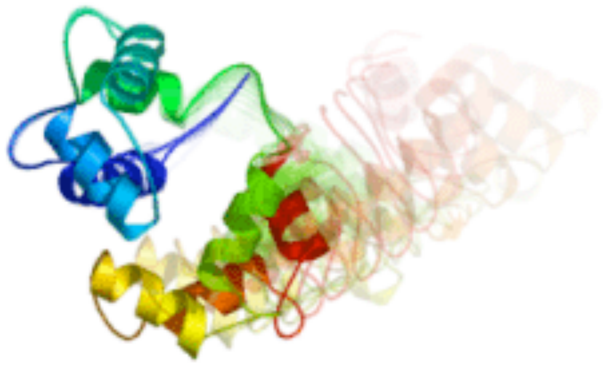
Lectures.GersteinLab.org

(See Last Slide for References & More Info.)

GersteinLab.org Research Overview: Bioinformatics

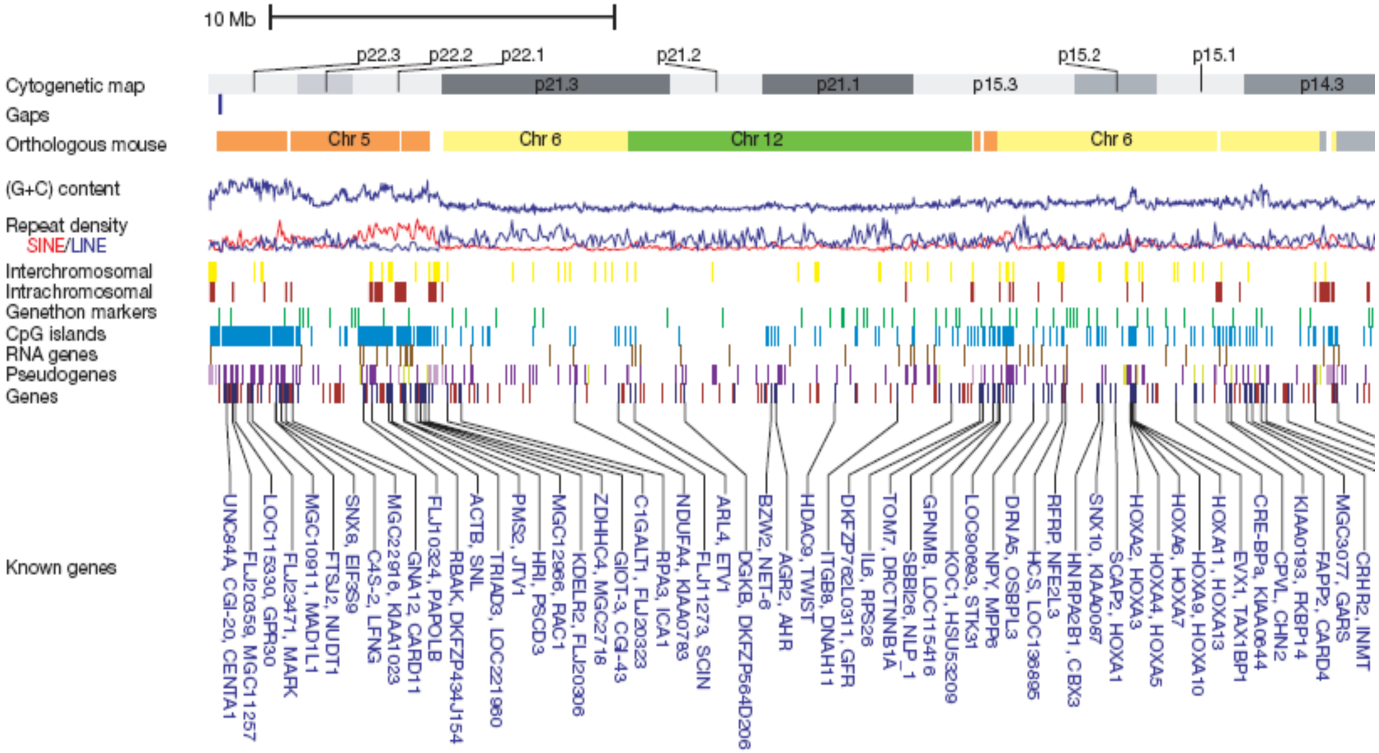
- **Genome Annotation**
  - ◊ Characterizing non-coding regions of the genome, focusing on protein fossils and novel RNAs (Pseudogene.org + GenomeTech.GersteinLab.org)
  - ◊ Personal Genomics – esp. related to SVs
- **Molecular Networks**
  - ◊ Using molecular networks to integrate & mine functional genomics information and describe genefunction on a large-scale (Networks.GersteinLab.org)
- **Macromolecular Motions**
  - ◊ Analyzing select populations of 3D-structures in detail, trying to understand their flexibility in terms of packing (MolMovDB.org)





The problem: Grappling with Function on a Genome Scale?

sequence of human chr. 7



~1,200 protein-coding genes

(~950 pseudogenes)

[Hillier et al, Nature, 424, 157]

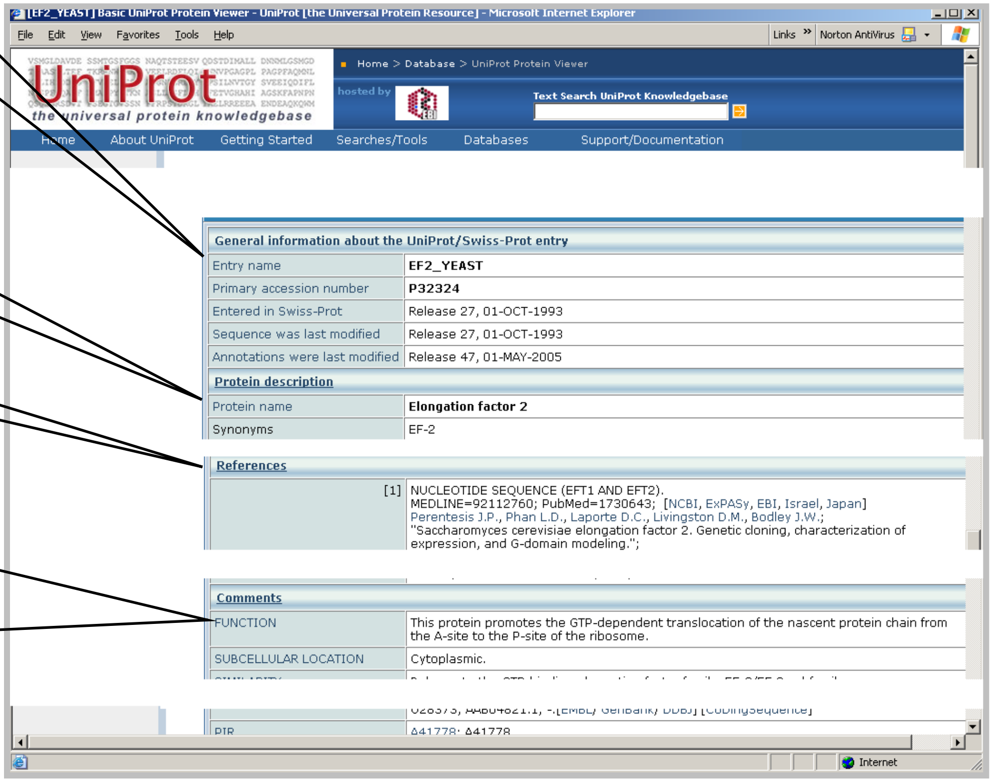
Traditional single molecule way to integrate evidence & describe function

EF2_YEAST

Descriptive Name:
Elongation Factor 2

Lots of references
to papers

Summary sentence describing function:
This protein promotes the GTP-dependent translocation of the nascent protein chain from the A-site to the P-site of the ribosome.


[EF2_YEAST] Basic UniProt Protein Viewer - UniProt [the Universal Protein Resource] - Microsoft Internet Explorer
File Edit View Favorites Tools Help
Links » Norton AntiVirus
UniProt
the universal protein knowledgebase
Home > Database > UniProt Protein Viewer
hosted by
Text Search UniProt Knowledgebase
Home
About UniProt
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Searches/Tools
Databases
Support/Documentation
Internet

General information about the UniProt/Swiss-Prot entry	
Entry name	**EF2_YEAST**
Primary accession number	**P32324**
Entered in Swiss-Prot	Release 27, 01-OCT-1993
Sequence was last modified	Release 27, 01-OCT-1993
Annotations were last modified	Release 47, 01-MAY-2005
Protein description	
Protein name	**Elongation factor 2**
Synonyms	EF-2

References

[1] NUCLEOTIDE SEQUENCE (EFT1 AND EFT2).
MEDLINE=92112760; PubMed=1730643; [NCBI, ExPASy, EBI, Israel, Japan]
Perentesis J.P., Phan L.D., Laporte D.C., Livingston D.M., Bodley J.W.;
"Saccharomyces cerevisiae elongation factor 2. Genetic cloning, characterization of expression, and G-domain modeling.";

Comments	
FUNCTION	This protein promotes the GTP-dependent translocation of the nascent protein chain from the A-site to the P-site of the ribosome.
SUBCELLULAR LOCATION	Cytoplasmic.
SIMILARITY	Belongs to the GTP-binding elongation factor family. EF-G/EF-2 subfamily.
	U28373; AAB04821.1; -.[EMBL/ GenBank/ DDBJ] [CodingSequence]
PIR	A41778; A41778

Some obvious issues in scaling single molecule definition to a genomic scale

- Fundamental complexities
  - ◊ Role Conflation:
    molecular, cellular, phenotypic
  - ◊ Often >2 proteins/function
  - ◊ Also Multi-functionality:
    2 functions/protein
    - phenotypically – e.g. Pleiotropic effects such as human PKU being involved in retardation & eczema
    - cellular role – e.g. Depending on the molecule it interacts with HSP70 is involved with protein folding, translocation of proteins into mitochondia, biogenesis of certain subunits..
    -

[HSP from Craig et al, Rev Physiol Biochem Pharmacol (2006) 156:1 ; Terms from Seringhaus et al. GenomeBiology (2008)]

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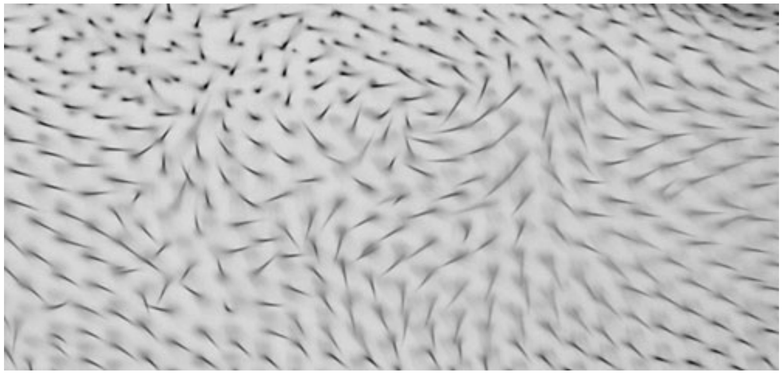
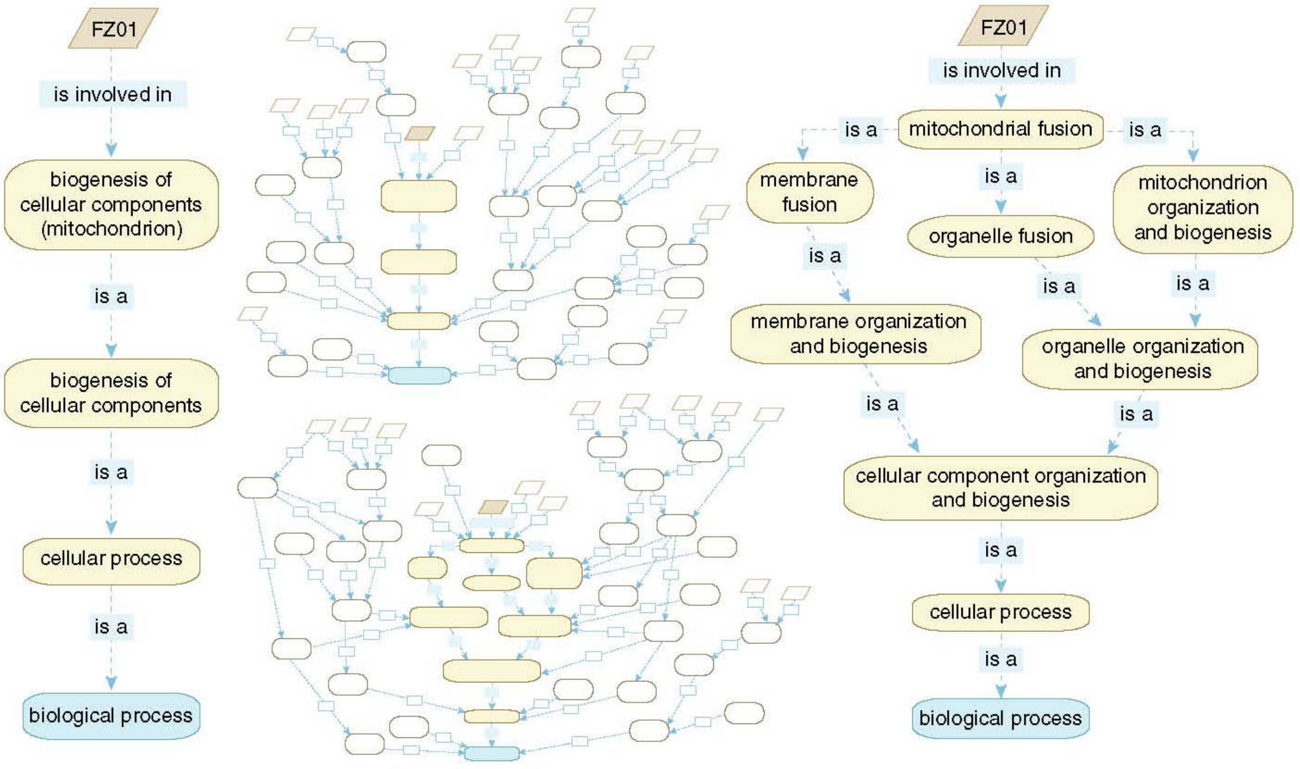
- Fun terms… but do they scale?....
  - **Starry night** (P Adler, ’94)



[HSP from Craig et al, Rev Physiol Biochem Pharmacol (2006) 156:1 ; Terms from Seringhaus et al. GenomeBiology (2008)]

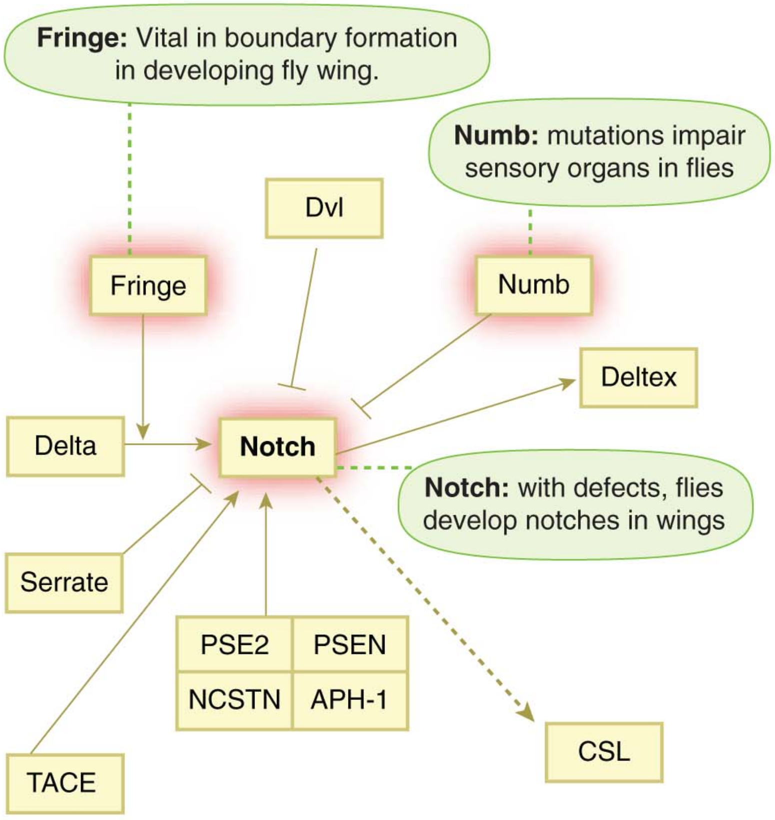
Hierarchies & DAGs of controlled-vocab terms but still have issues...


FZ01
is involved in
biogenesis of cellular components (mitochondrion)
is a
biogenesis of cellular components
is a
cellular process
is a
biological process
FZ01
is involved in
mitochondrial fusion
is a
membrane fusion
is a
organelle fusion
is a
mitochondrion organization and biogenesis
is a
membrane organization and biogenesis
is a
organelle organization and biogenesis
is a
cellular component organization and biogenesis
is a
cellular process
is a
biological process

MIPS (Mewes et al.) **GO (Ashburner et al.)**

[Seringhaus & Gerstein, Am. Sci. '08]

Networks (Old & New)

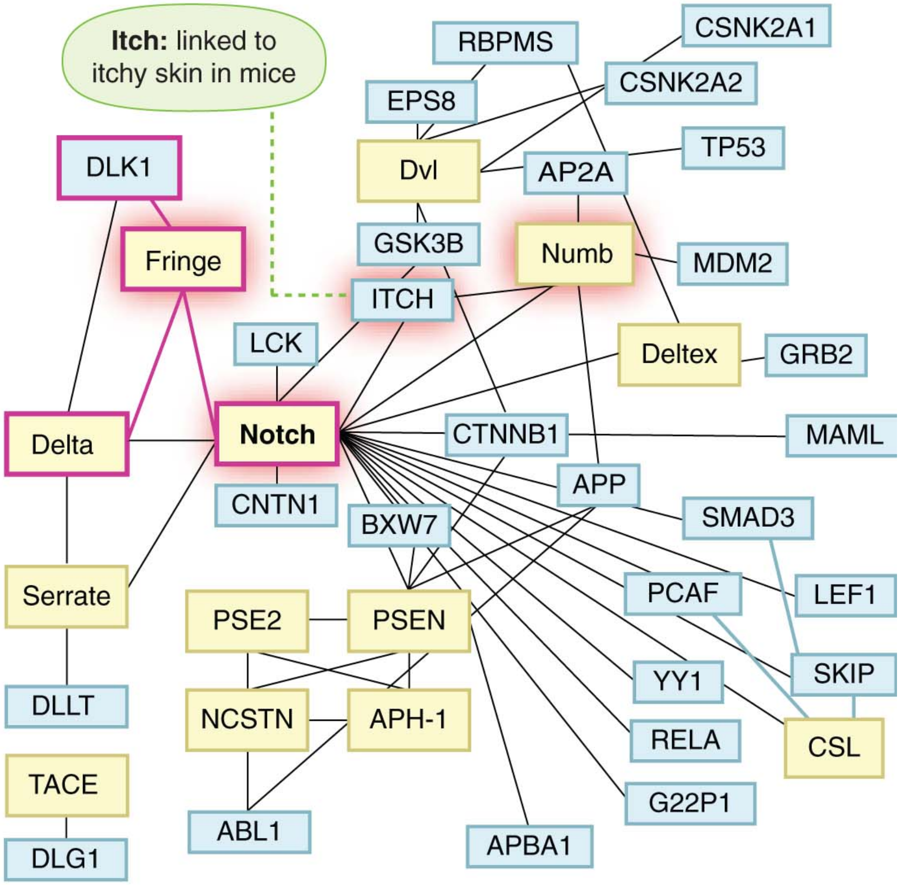


Fringe: Vital in boundary formation in developing fly wing.

Numb: mutations impair sensory organs in flies

Notch: with defects, flies develop notches in wings

Classical KEGG pathway

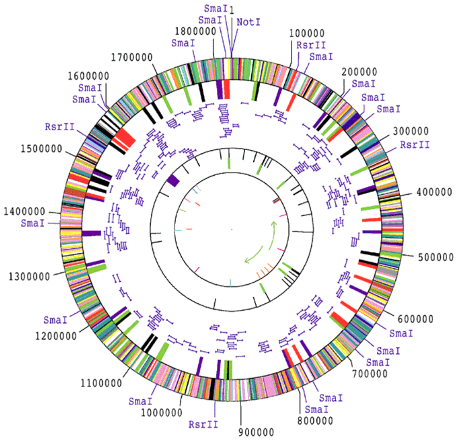


Itch: linked to itchy skin in mice

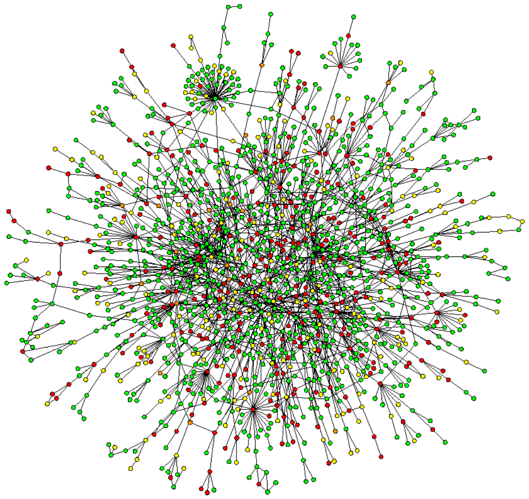
Same Genes in High-throughput Network

[Seringhaus & Gerstein, Am. Sci. '08]

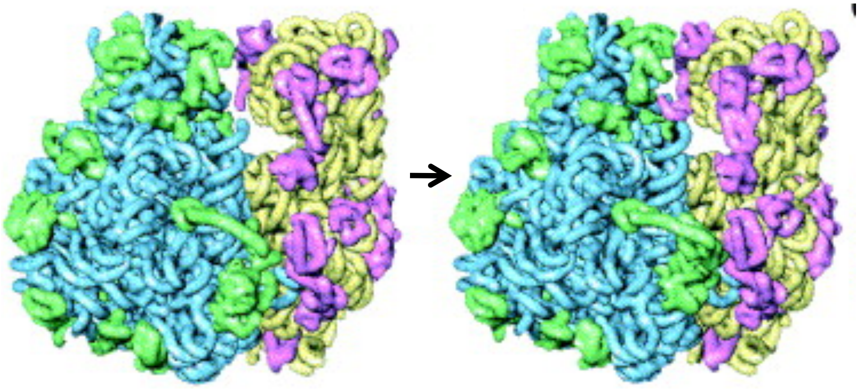
Networks occupy a midway point in terms of level of understanding



1D: Complete
Genetic Partslist



~2D: Bio-molecular
Network
Wiring Diagram



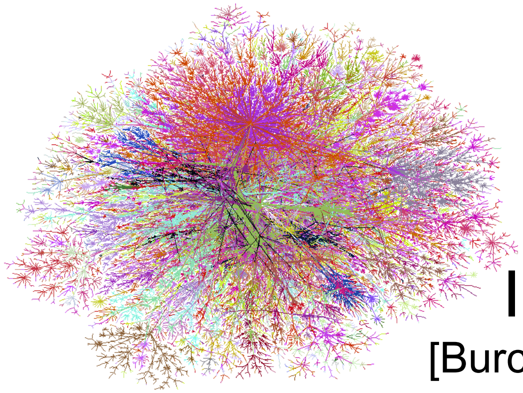
3D and 4D:
Detailed structural understanding
of cellular machinery
(e.g. ribosome in different
functional states)

[Fleischmann et al., Science, 269 :496]

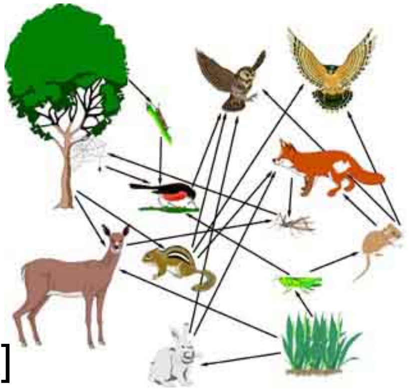
[Jeong et al. Nature, 41:411]

[Chiu et al. Trends in Cell Biol, 16:144]

Networks as a universal language

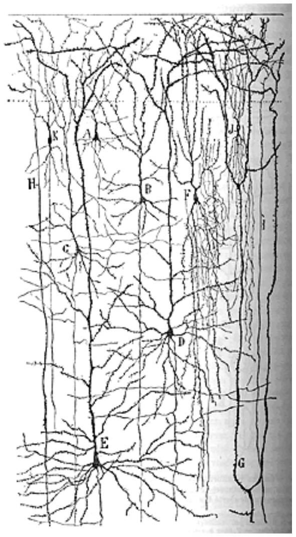


Internet
[Burch & Cheswick]

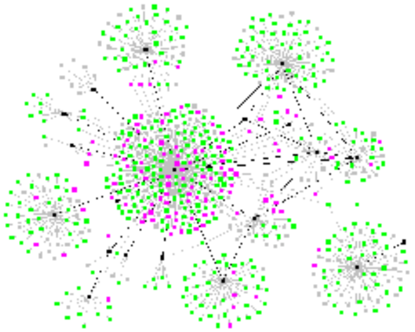


Food Web

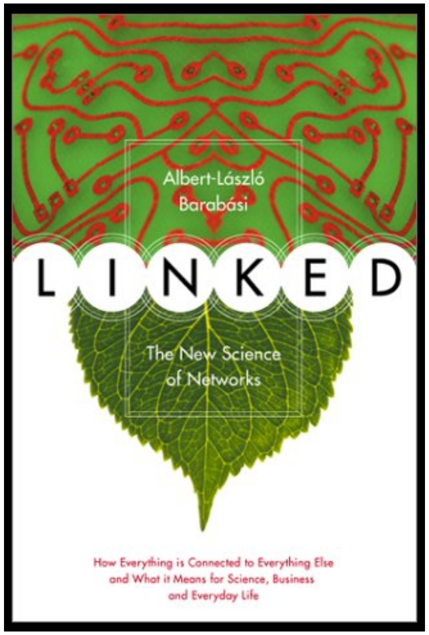
Electronic Circuit



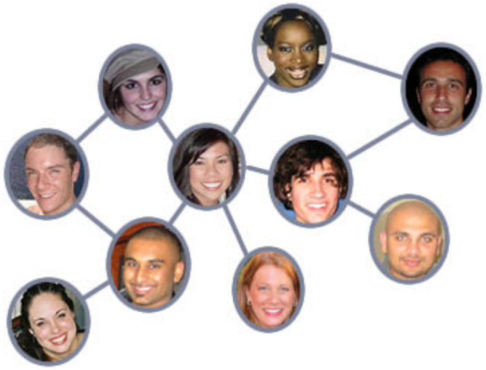
Neural Network
[Cajal]



Disease Spread
[Krebs]

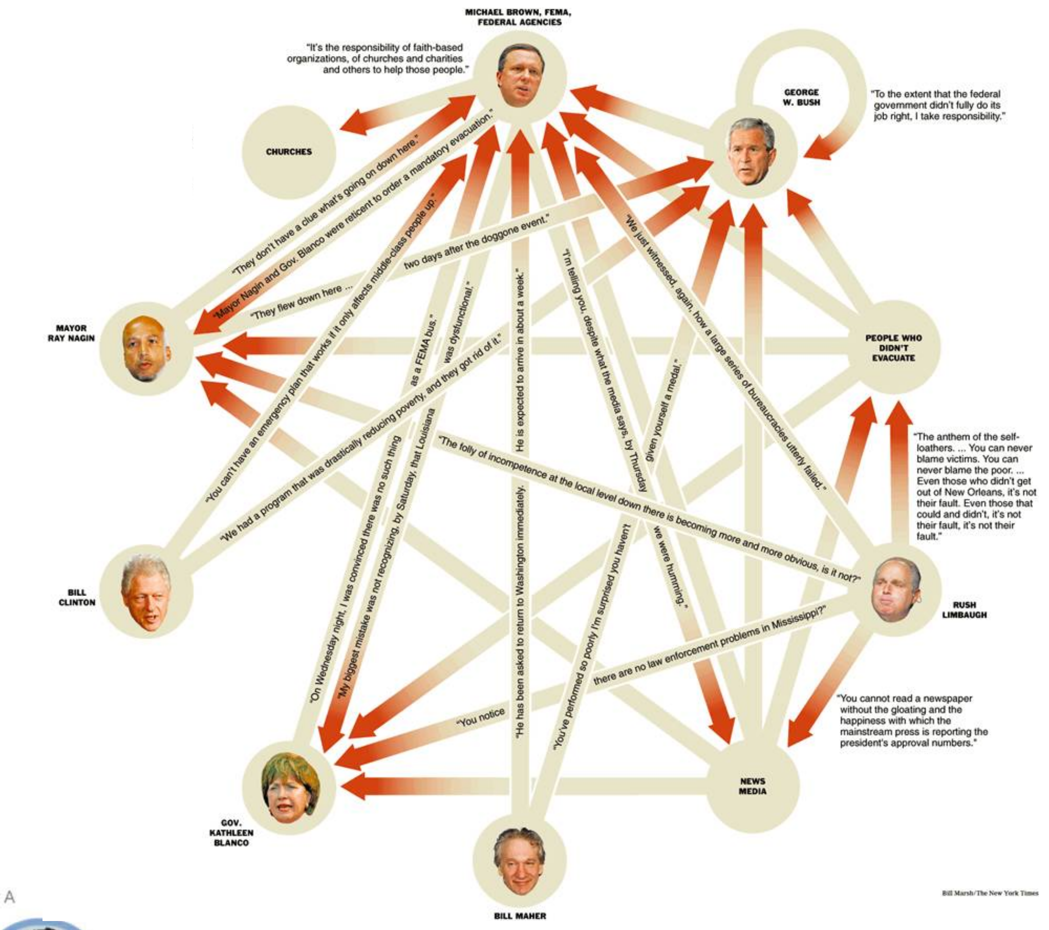

Albert-László Barabási
LINKED
The New Science of Networks
How Everything is Connected to Everything Else and What it Means for Science, Business and Everyday Life

Protein Interactions
[Barabasi]



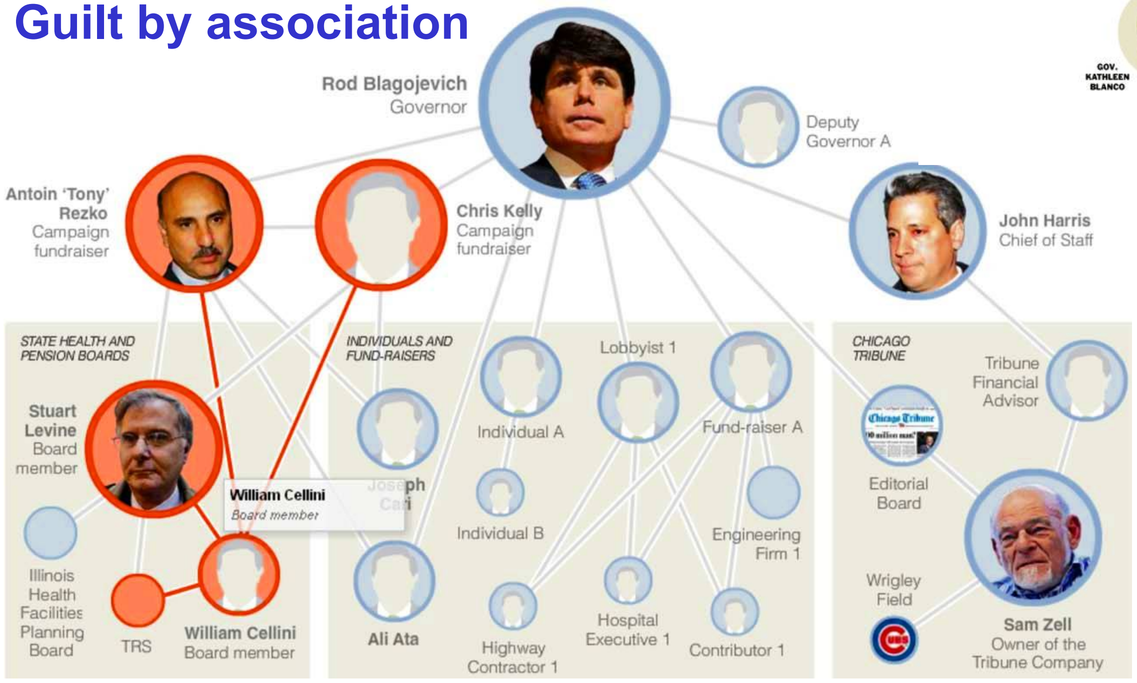
Social Network

Using the position in networks to describe function


MICHAEL BROWN, FEMA, FEDERAL AGENCIES
"It's the responsibility of faith-based organizations, of churches and charities and others to help those people."
CHURCHES
GEORGE W. BUSH
"To the extent that the federal government didn't fully do its job right, I take responsibility."
MAYOR RAY NAGIN
"They don't have a clue what's going on down here."
"Mayor Nagin and Gov. Blanco were reticent to order a mandatory evacuation."
"They flew down here ... two days after the doggone event."
"You can't have an emergency plan that works if it only affects middle-class people up."
"We had a program that was drastically reducing poverty, and they got rid of it."
"On Wednesday night, I was convinced there was no such thing as a FEMA bus."
"My biggest mistake was not recognizing, by Saturday, that Louisiana was dysfunctional."
"He has been asked to return to Washington immediately. He is expected to arrive in about a week."
"I'm telling you, despite what the media says, by Thursday we were humming."
"You've performed so poorly I'm surprised you haven't given yourself a medal."
"We just witnessed, again, how a large series of bureaucracies utterly failed."
"The folly of incompetence at the local level down there is becoming more and more obvious, is it not?"
"You notice there are no law enforcement problems in Mississippi?"
PEOPLE WHO DIDN'T EVACUATE
"The anthem of the self-loathers. ... You can never blame victims. You can never blame the poor. ... Even those who didn't get out of New Orleans, it's not their fault. Even those that could and didn't, it's not their fault, it's not their fault."
RUSH LIMBAUGH
"You cannot read a newspaper without the gloating and the happiness with which the mainstream press is reporting the president's approval numbers."
BILL CLINTON
GOV. KATHLEEN BLANCO
BILL MAHER
NEWS MEDIA

Bill Marsh/The New York Times

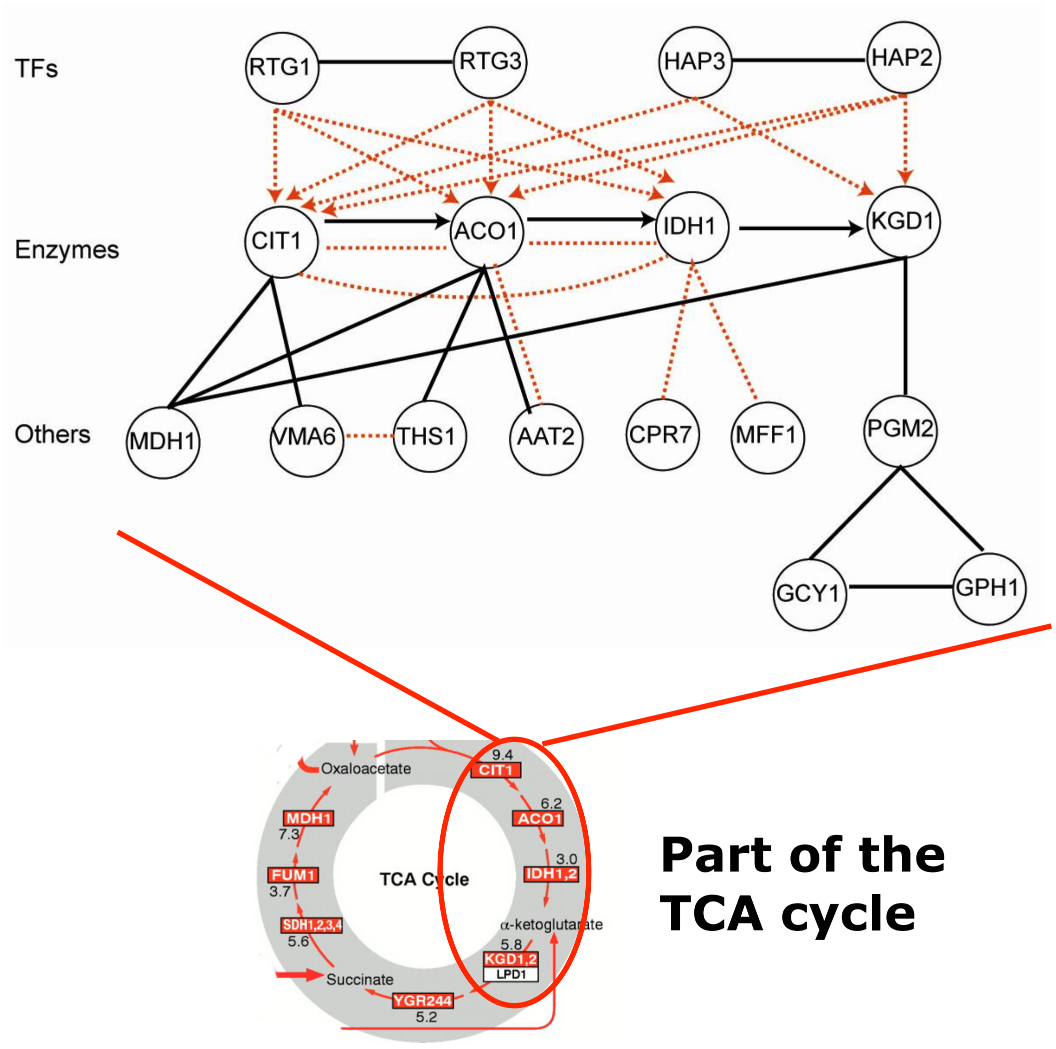
Guilt by association



Finding the causal regulator (the "Blame Game")

[NY Times, 2-Oct-05, 9-Dec-08]

Combining networks forms an ideal way of integrating diverse information



Part of the TCA cycle

Metabolic pathway

Transcriptional regulatory network

Physical protein-protein Interaction

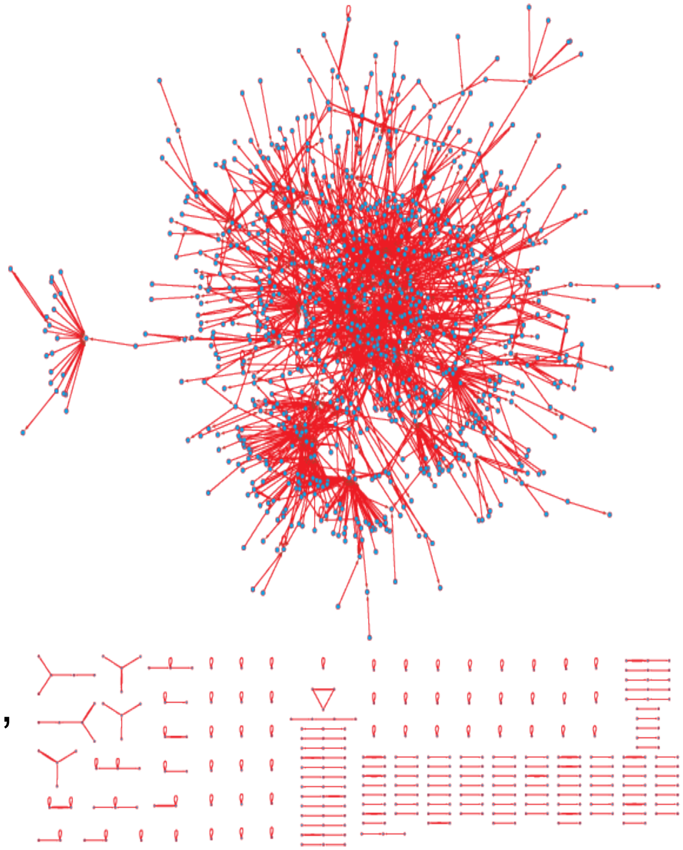
Co-expression Relationship

Genetic interaction (synthetic lethal)
Signaling pathways

Outline: Molecular Networks

- Why Networks?
- Generating Networks
  - ◊ Processing Protein Chips
    (yeast & human nets)
  - ◊ Propagating Known Information
    (yeast ppi)
- Central Points in Networks
  - ◊ Hubs & Bottlenecks
    (yeast ppi & reg. net)
  - ◊ Tops of Heirarchies
    (yeast reg. net)
  - ◊ Identified by score
    (human miRNA-targ. net)
- Dynamics of Networks
  - ◊ Across environments
    (prokaryote metab. pathways)
- Protein Networks & Variation
  (human ppi & miRNA-targ. net)

Example: yeast PPI network



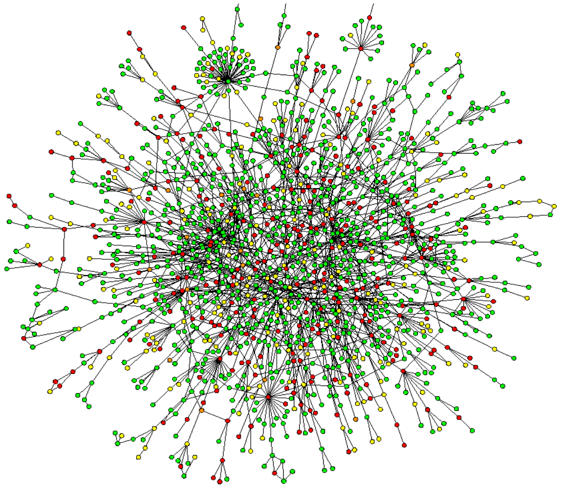
Actual size:

- ◊ ~6,000 nodes
  → Computational cost: ~18M pairs
- ◊ Estimated ~15,000 edges
  → Sparseness: 0.08% of all pairs (Yu et al., 2008)

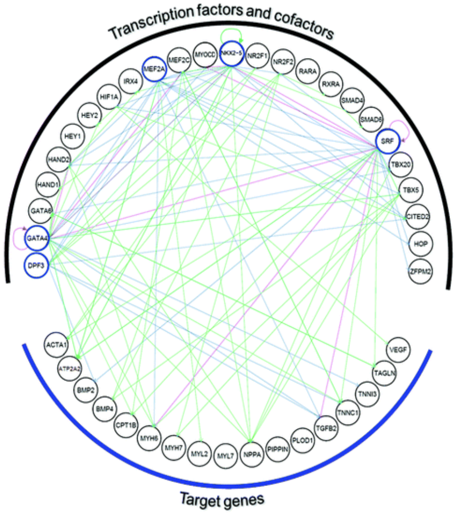
Known interactions:

- ◊ Small-scale experiments: accurate but few
  → Overfitting: ~5,000 in BioGRID, involving ~2,300 proteins
- ◊ Large-scale experiments: abundant but noisy
  → Noise: false +ve/-ve for yeast two-hybrid data up to
  45% and 90% (Huang et al., 2007)

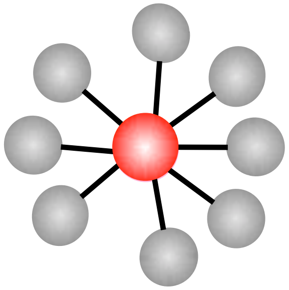
Different Types of Molecular Networks



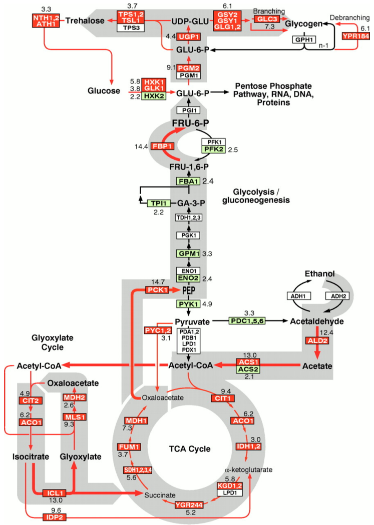
Protein-protein Interaction networks



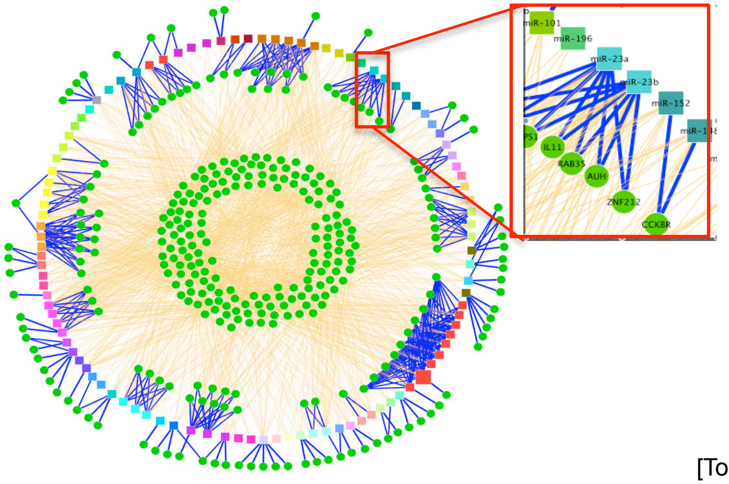
TF-target-gene Regulatory networks



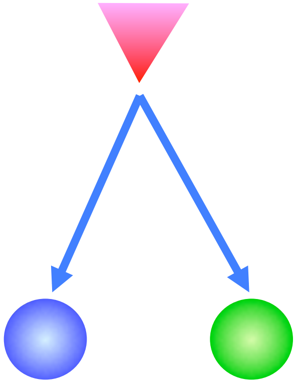
Undirected



Metabolic pathway networks



miRNA-target networks



Directed

[Toenjes, *et al*, *Mol. BioSyst.* (2008); Jeong *et al*, *Nature* (2001); [Horak, et al, Genes & Development, 16:3017-3033; DeRisi, Iyer, and Brown, Science, 278:680-686]

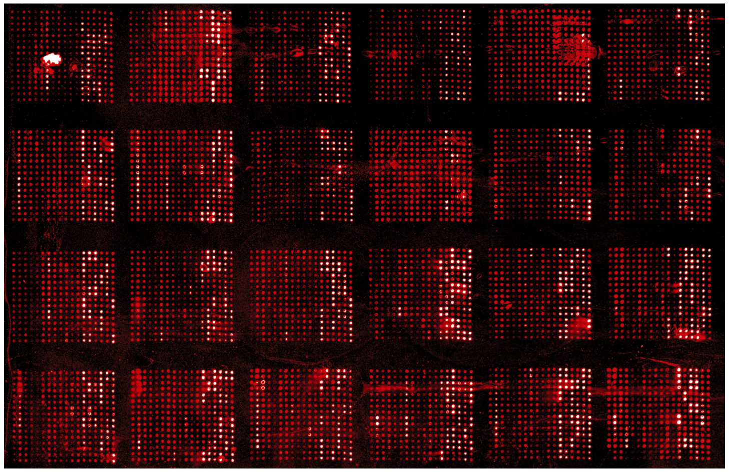
Generating Networks

How do we construct large molecular networks. From processing high-throughput protein array data?

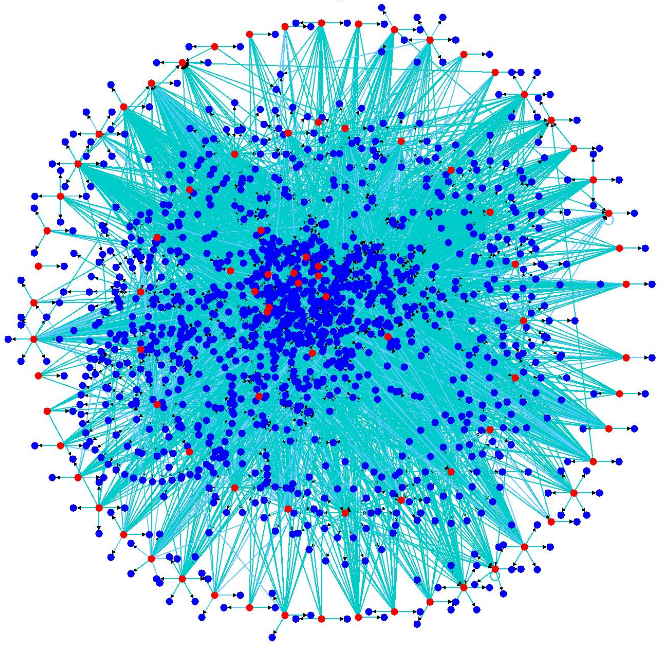
Protein Networks from Processing Protein Chip Data

- Array functional proteins on a chip
- Readout can show presence of proteins in sera (via autoantibodies), small mol. interactions, enzymatic activity, & **protein interactions**

- Technical issues in processing protein chips similar but not identical to those for DNA chips
  - ◊ Hybridization v protein binding
  - ◊ Background correction & denoising, Normalizing across chips & replicates, Calling "hits"
  - ◊ ProCAT (Zhu et al., GenomeBiology, '06) & **RLM (Sboner et al., J Proteome Sci. '09)**



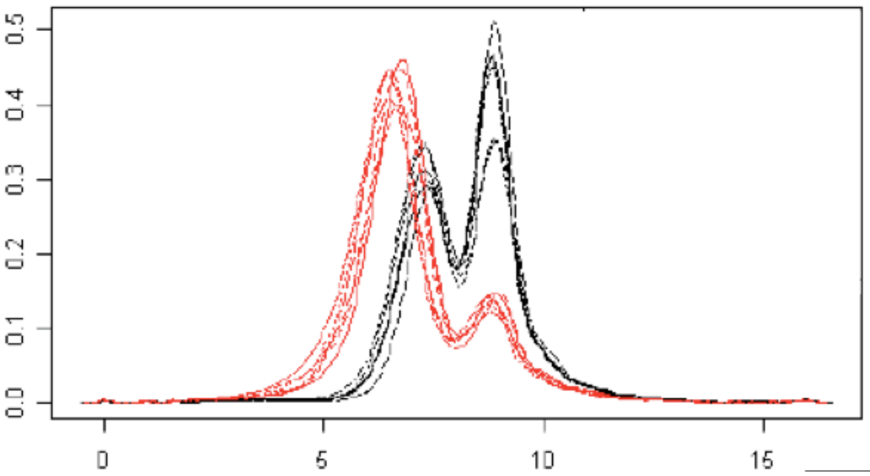
~6000 yeast proteins on a chip, Zhu et al. Science ('01)



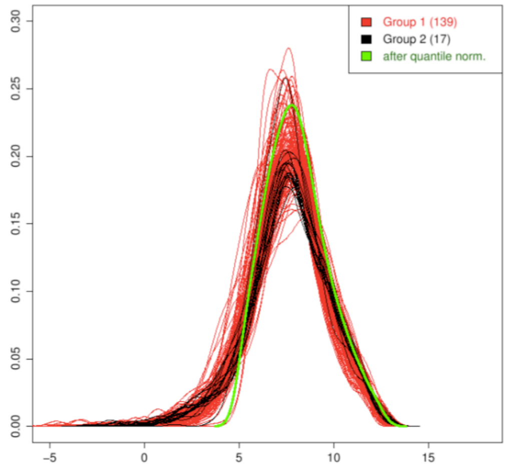
4200 phosphorylations involving 1325 proteins, Ptacek et al. Nature ('05)

Signal Distribution & Metrics

[Sboner et al.,
J Proteome Sci. '09]

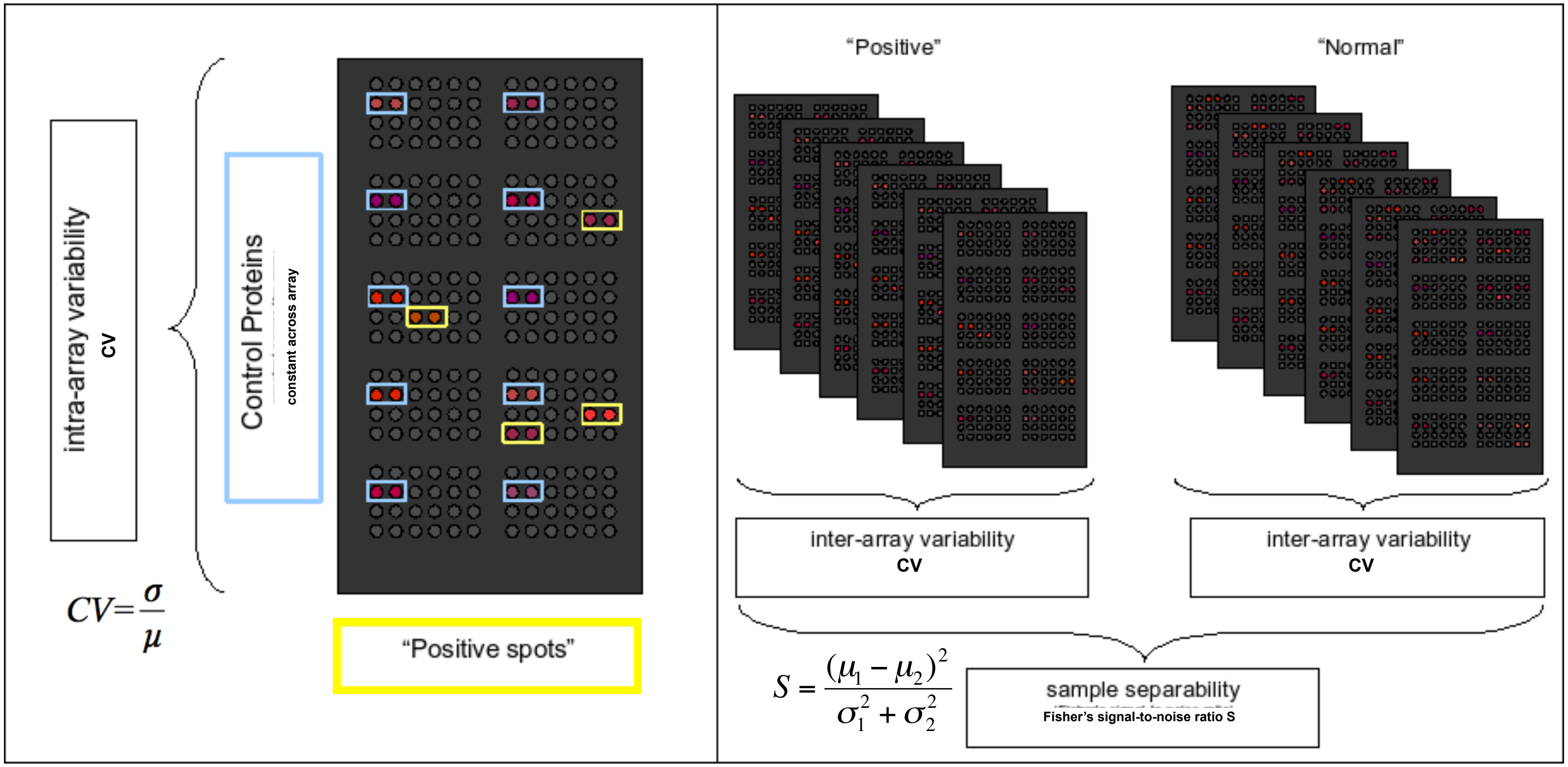


Protein Chip Sig. Intensity Distribution
from different applied sera
(**NEG**ative & with **POS**itive sera)



Representative DNA
Chip Sig. Dist.

Goal: Decreasing variation betw. replicates (both inter- & intra- array), measured by **CV**, & increasing **separability (S)** betw. known positive & negative samples



intra-array variability
CV

Control Proteins
constant across array

"Positive spots"

$$CV = \frac{\sigma}{\mu}$$

"Positive"

"Normal"

inter-array variability
CV

inter-array variability
CV

$$S = \frac{(\mu_1 - \mu_2)^2}{\sigma_1^2 + \sigma_2^2}$$

sample separability
Fisher's signal-to-noise ratio S

RLM Normalization, how it compares?

NORMALIZATION

– **Global**

- A single scaling factors

– **Quantile**

- Signals are normalized robustly according to the quantiles of a reference distribution

– **Robust Linear Model** **RLM**

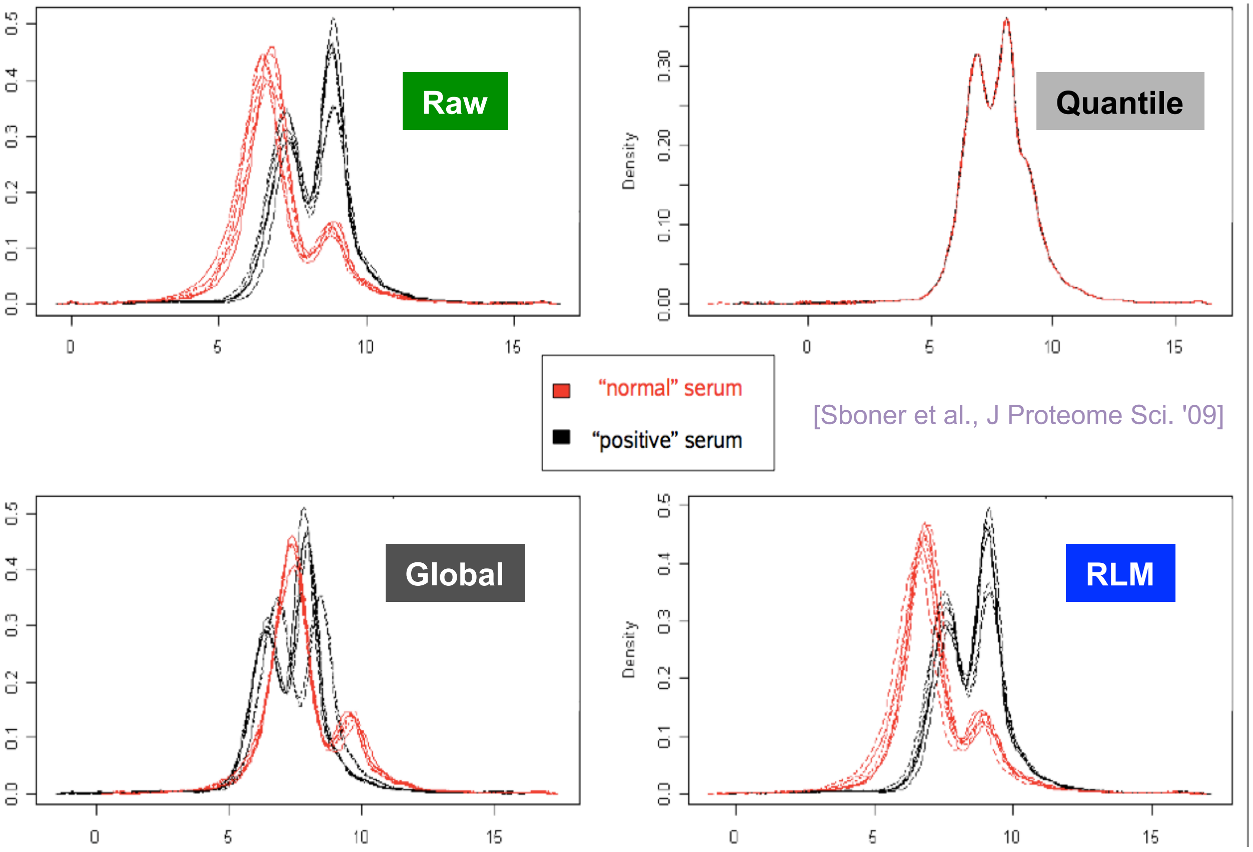
$$y_{ijkr} = \alpha_i + \beta_j + \tau_k + \varepsilon_{ijkr}$$

α_i **Slide-effect (inter slide)**

β_j **Sub-array effect (intra slide)**

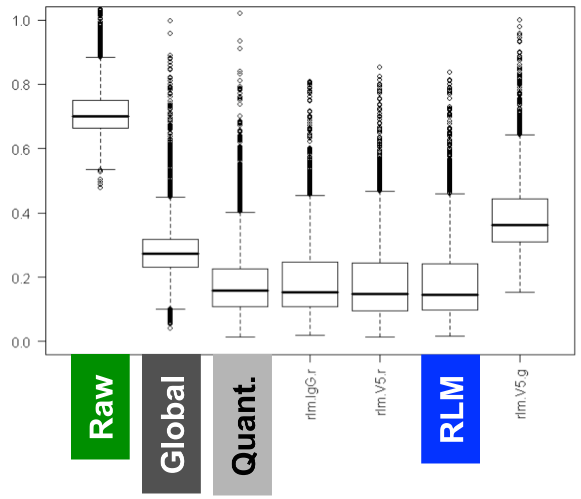
τ_k **Signal**

ε_{ijkr} **Random error**

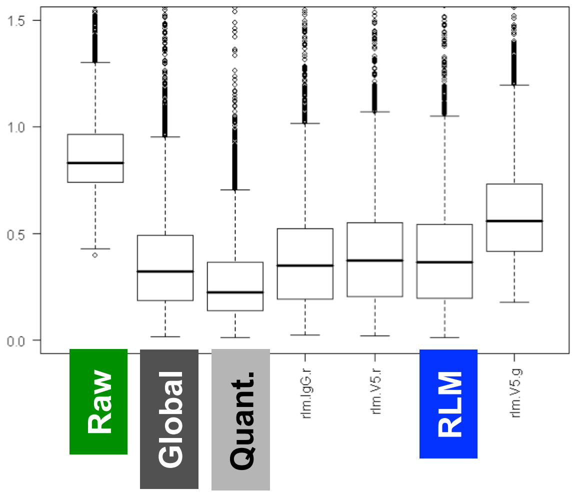


[Sboner et al., J Proteome Sci. '09]

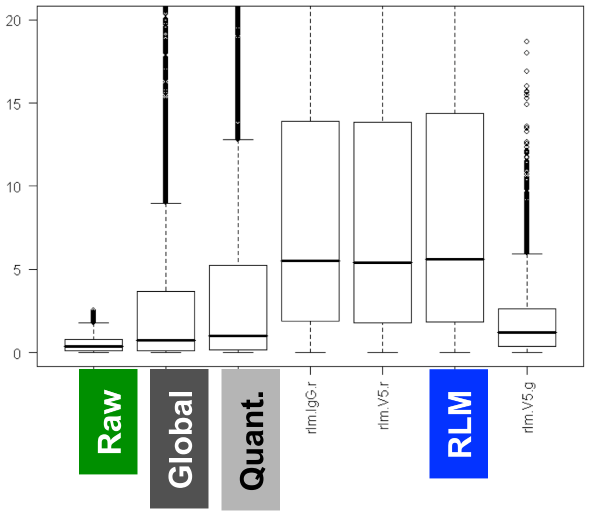
Inter-array CV: "positive" serum



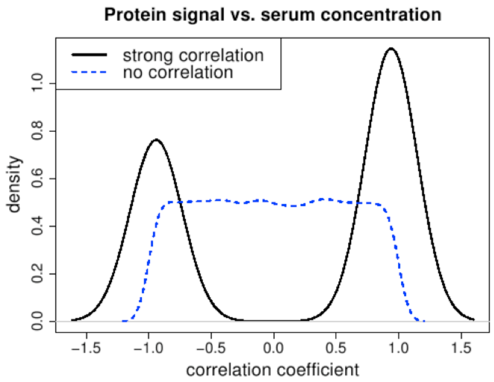
Inter-array CV: "normal" serum

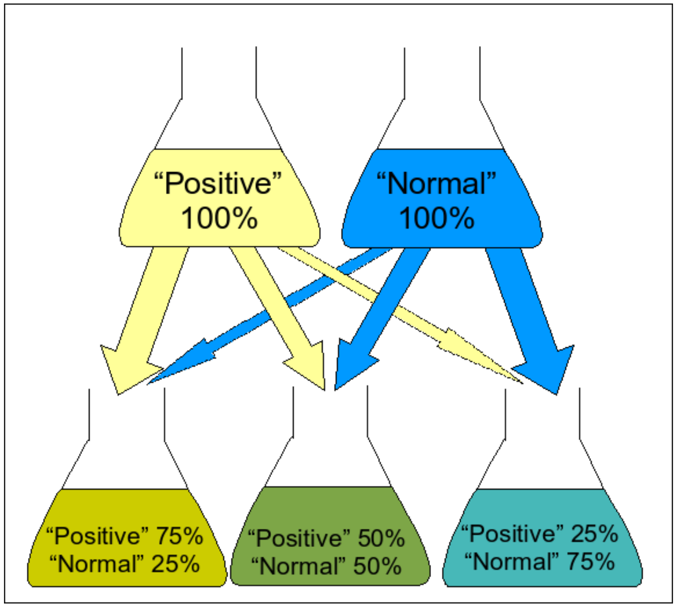


SAMPLE SEPARABILITY

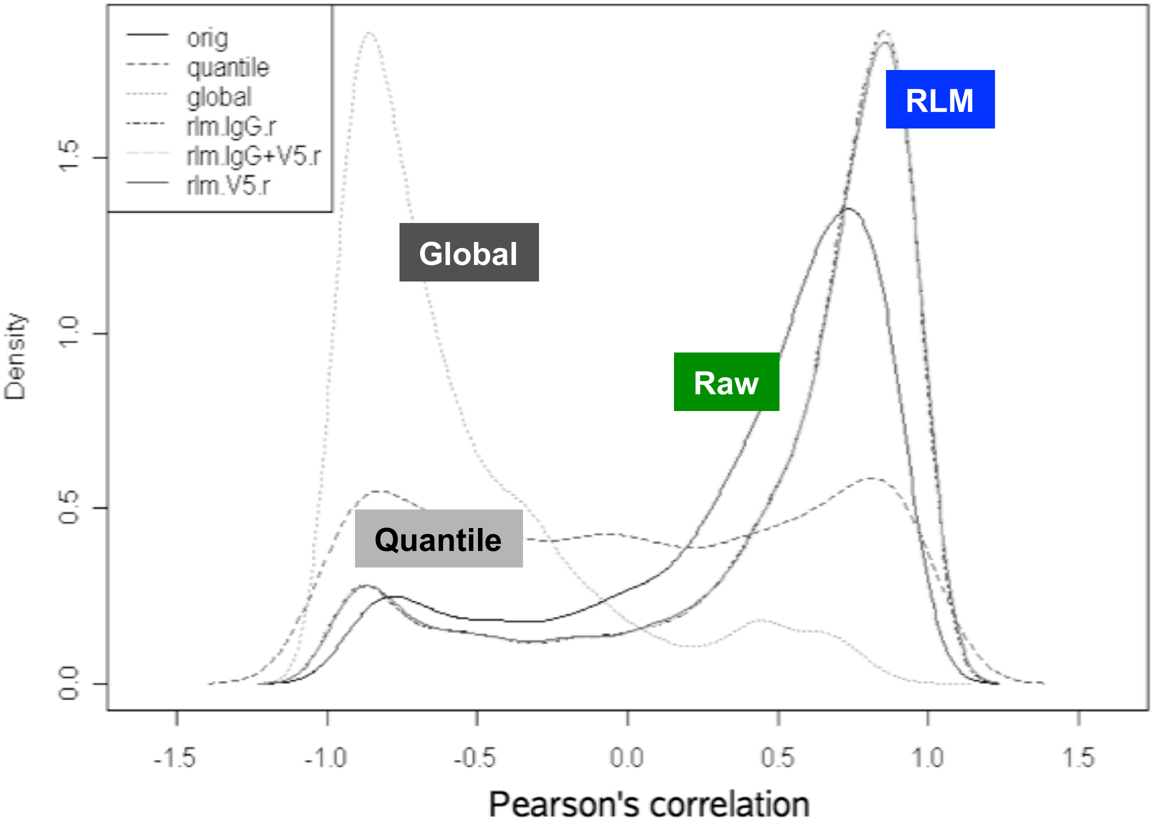


Check #2: How Signal Intensity Correlates over a Titration




"Positive" 100%
"Normal" 100%
"Positive" 75% "Normal" 25%
"Positive" 50% "Normal" 50%
"Positive" 25% "Normal" 75%

Correlation of signal intensity with "positive" serum concentration



Expectation

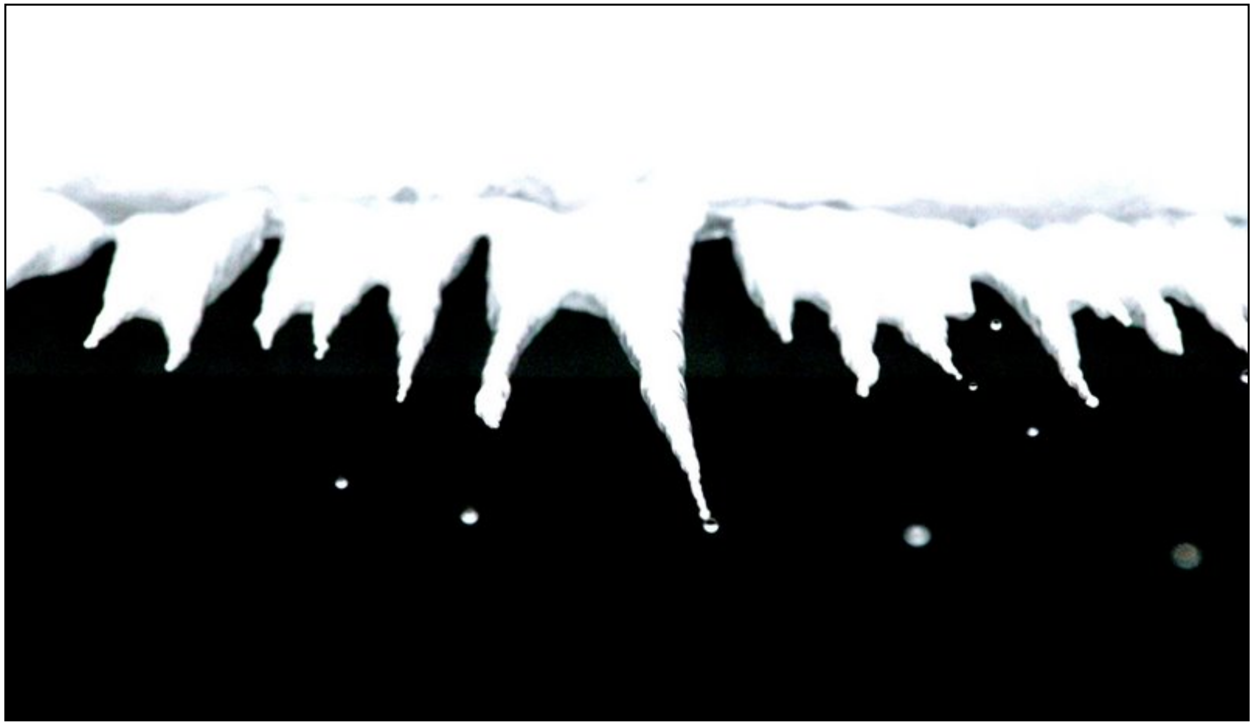
"Positive" protein signal should positively correlate with "Positive" serum dilution

Higher number of "hits" for the "Positive" serum

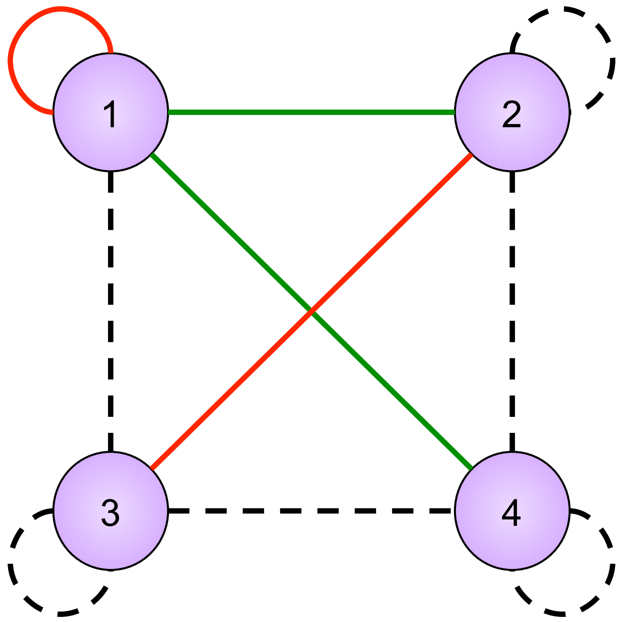
[Sboner et al., J Proteome Sci. '09]

Generating Networks #2

How do we construct large molecular networks?
From extrapolating correlations between functional genomics data with fairly small sets of known interactions, making best use of the known training data.



Training sets



	1	2	3	4
1	0	1	?	1
2	1	?	0	?
3	?	0	?	?
4	1	?	?	?

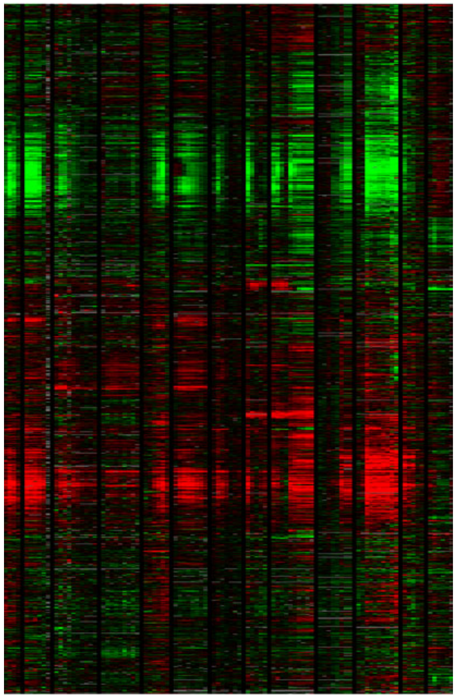
🟩 **Known interactions**

🟥 **Known non-interactions**

- - - **Unknown**

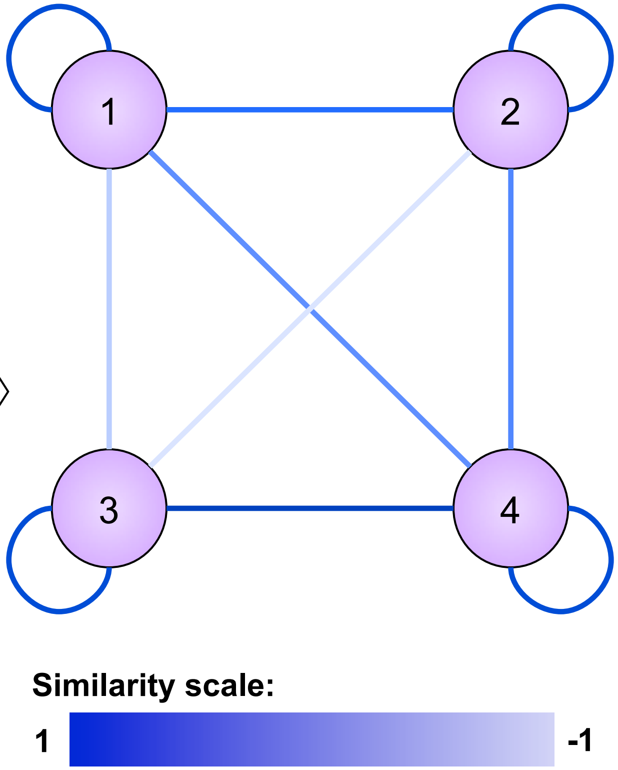
Network prediction: features

- Example 1: gene expression



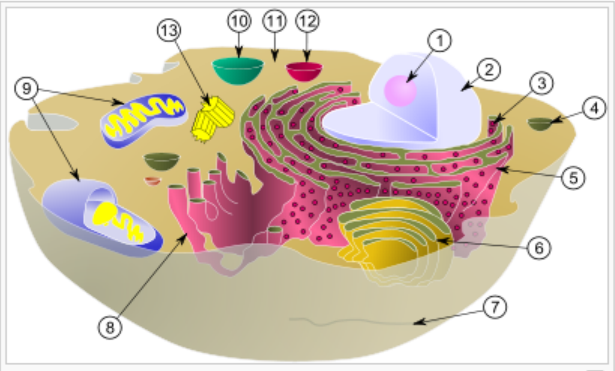
Gasch et al., 2000

$x_1 = (0.2, 2.4, 1.5, \ldots)$
$x_2 = (0.8, 2.2, 1.5, \ldots)$
$x_3 = (4.3, 0.1, 7.5, \ldots)$
$\ldots$
$\mathrm{sim}(x_1, x_2) = 0.62$
$\mathrm{sim}(x_1, x_3) = -0.58$
$\ldots$



Network prediction: features

- Example 2: sub-cellular localization



http://www.scq.ubc.ca/wp-content/yeasttwohybridtranscript.gif

$x_1 = (1, 1, 0, 0, \ldots)$

$x_2 = (1, 1, 1, 0, \ldots)$

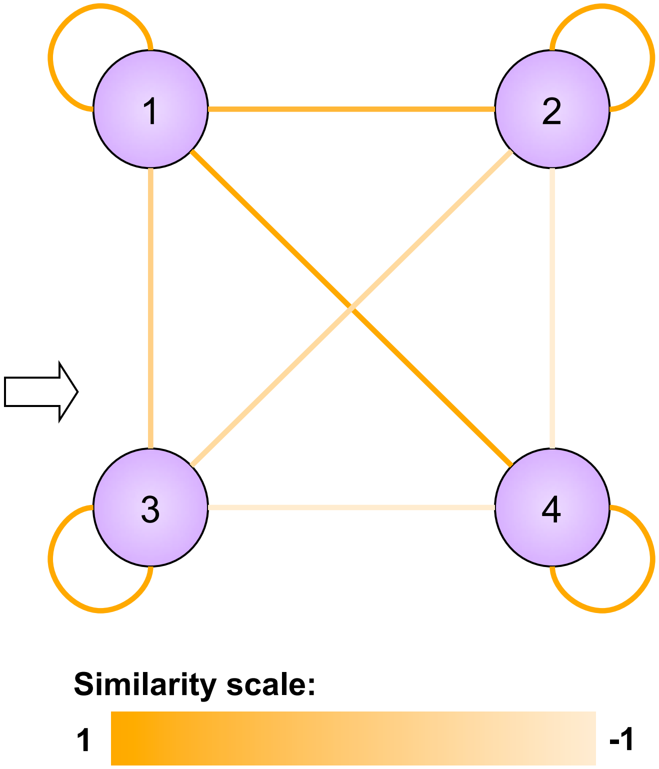
$x_3 = (1, 0, 1, 0, \ldots)$

$\ldots$

$\mathrm{sim}(x_1, x_2) = 0.81$

$\mathrm{sim}(x_1, x_3) = 0.12$

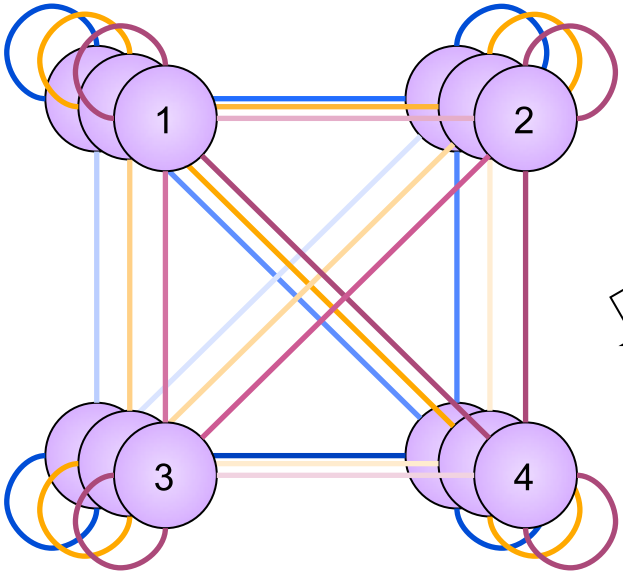
$\ldots$

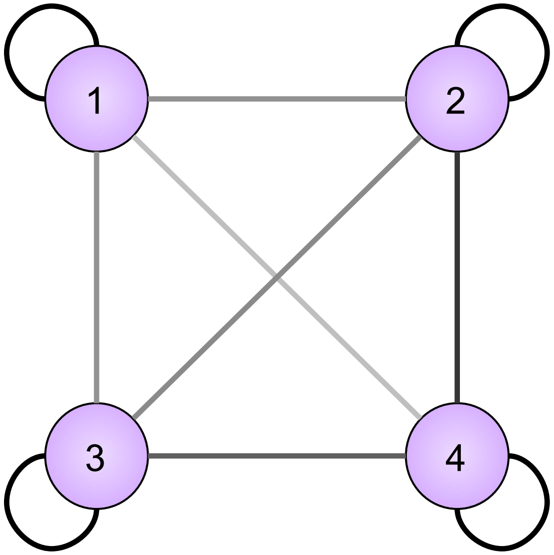


Similarity scale:

1 -1

Data integration & Similarity Matrix





	1	2	3	4
1	1.00	0.57	0.55	0.40
2	0.57	1.00	0.66	0.89
3	0.55	0.66	1.00	0.79
4	0.40	0.89	0.79	1.00

Learning methods

An endless list:

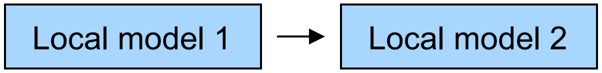
- Docking (e.g. Schoichet and Kuntz 1991)
- Evolutionary (e.g. Ramani and Marcotte, 2003)
- Topological (e.g. Yu et al., 2006)
- Bayesian (e.g. Jansen et al., 2003)
- Kernel methods
  - ◊ Global modeling:
    - em (Tsuda et al., 2003)
    - kCCA (Yamanishi et al., 2004)
    - kML (Vert and Yamanishi, 2005)
    - Pairwise kernel (Pkernel) (Ben-Hur and Noble, 2005)
  - ◊ Local modeling:
    - Local modeling (Bleakley et al., 2007)

Let's compare in a public challenge!
(DREAM: Dialogue for Reverse Engineering Assessment and Methods)

Our work: efficiently propagating known information

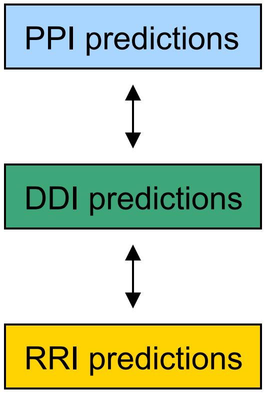
Training set expansion

- Motivation: lack of training examples
- Expand training sets horizontally



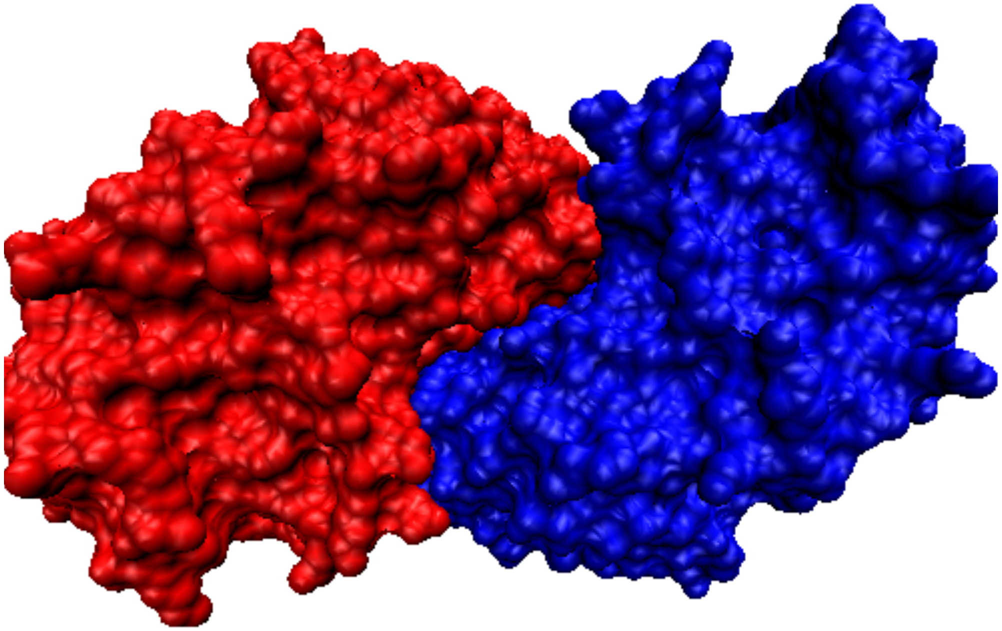
Multi-level learning

- Motivation: hierarchical nature of interaction
- Expand training sets vertically



DREAM3 *in silico* regulatory network reconstruction challenge

Protein interaction

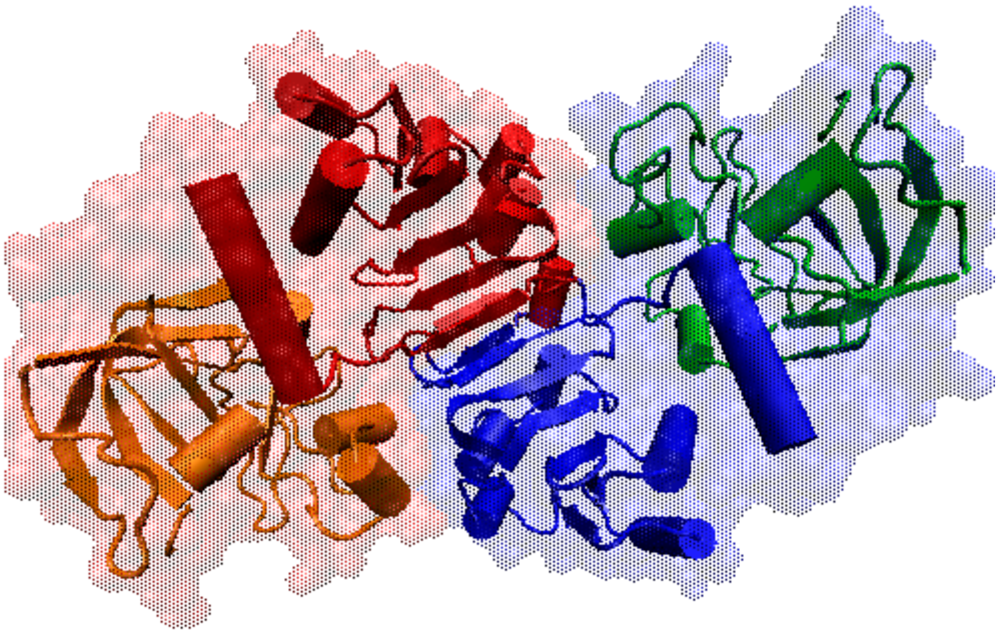


Yeast NADP-dependent alcohol dehydrogenase 6 (PDB: 1piw)

Protein-level features for interaction prediction: functional genomic information

[Yip and Gerstein, BMC Bioinfo. ('09, press)]

Domain interaction

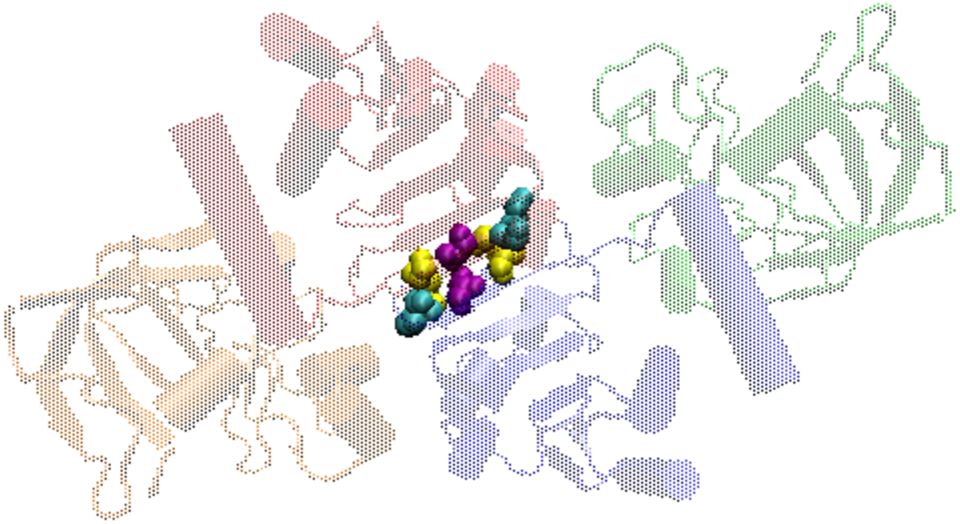


Pfam domains: PF00107 (inner) and PF08240 (outer)

Domain-level features for interaction prediction: evolutionary information

[Yip and Gerstein, BMC Bioinfo. ('09, press)]

Residue interaction

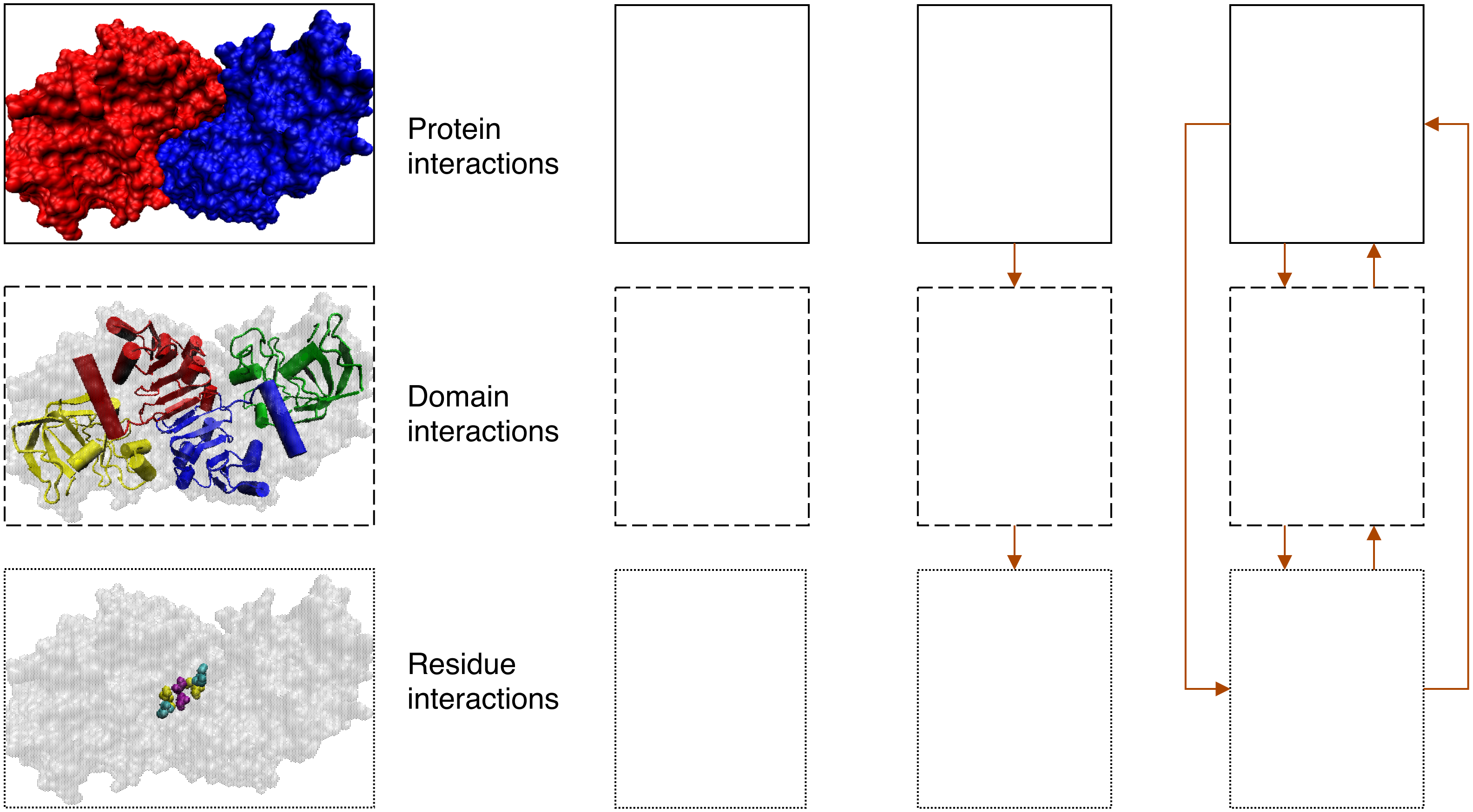


Interacting residues: 283 (yellow) with 287 (cyan), and 285 (purple) with 285

Residue-level features for interaction prediction: physical-chemical information

[Yip and Gerstein, BMC Bioinfo. ('09, press)]

Combining the three problems



Protein interactions

Domain interactions

Residue interactions

i. Independent levels

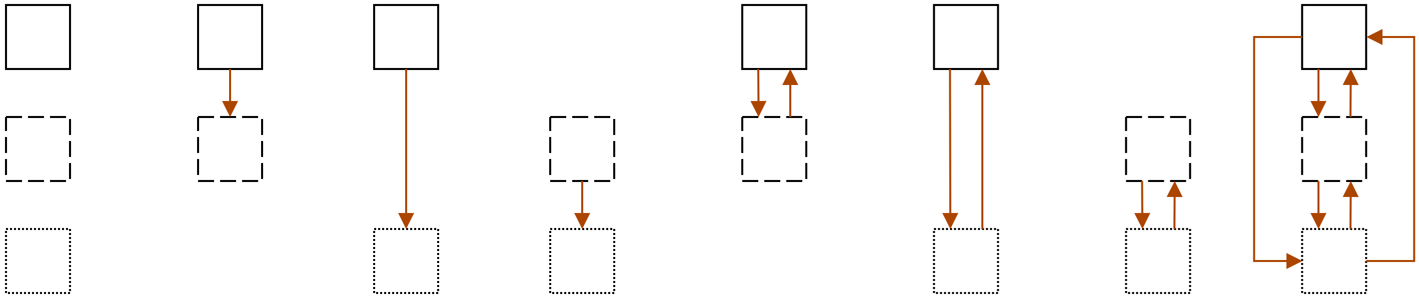
ii. Unidirectional flow

iii. Bidirectional flow

[Yip and Gerstein, BMC Bioinfo. ('09, press)]

Empirical results (AUCs)

	Ind. levels	Unidirectional flow			Bidirectional flow			
Level		PD	PR	DR	PD	PR	DR	PDR
Proteins	71.68				72.23	72.50		**72.82**
Domains	53.18	61.51			**71.71**		68.94	71.20
Residues	57.36		54.89	53.81		72.26	63.16	**77.86**



- Highest accuracy by bidirectional flow
- Additive effect: 2 vs. 3 levels

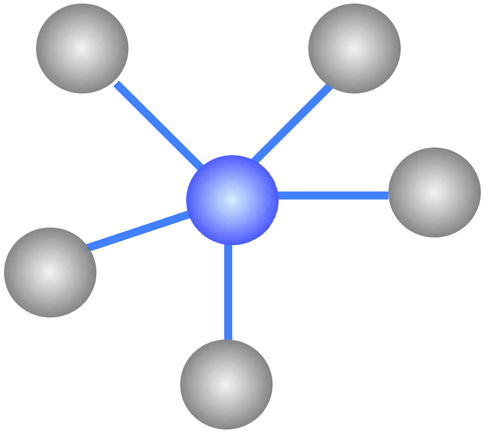
[Yip and Gerstein, BMC Bioinfo. ('09, press)]

Finding Central Points in Networks: Hubs & Bottlenecks

Where are key points networks ? How do we locate them ?

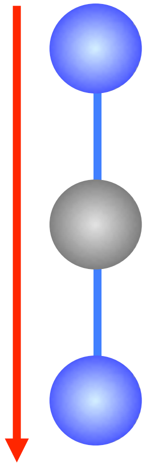
Global topological measures

Indicate the gross topological structure of the network



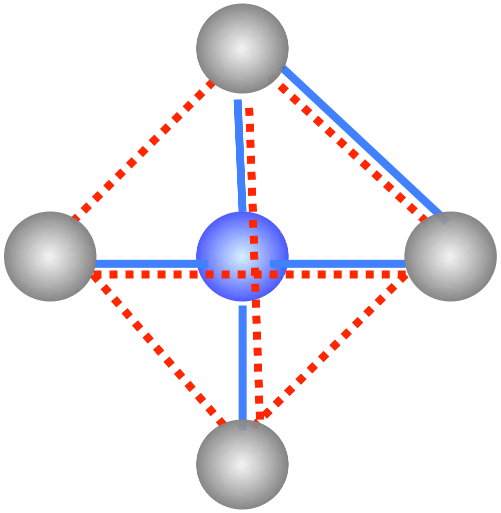
Degree (*K*)

5



Path length (*L*)

2



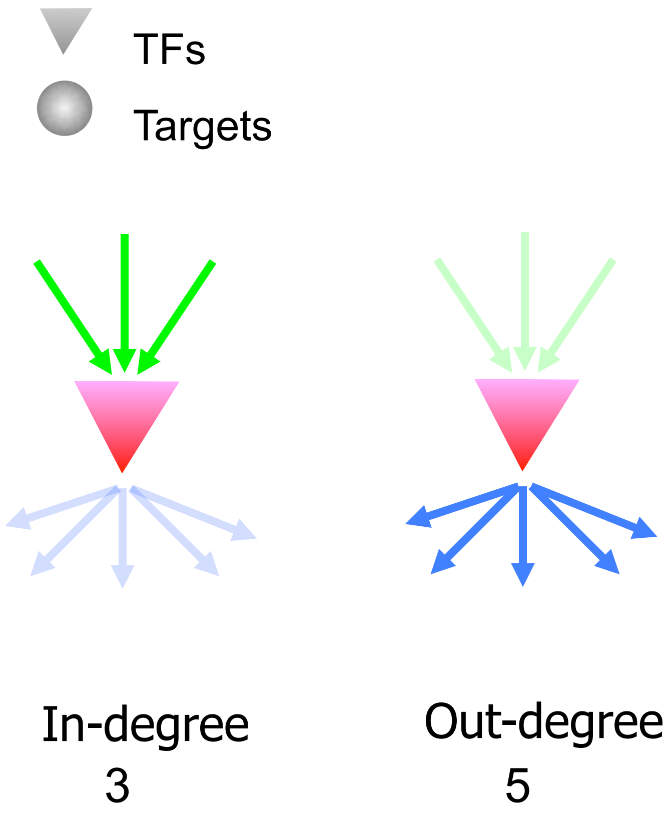
Clustering coefficient (*C*)

1/6

Interaction and expression networks are ***undirected***

[Barabasi]

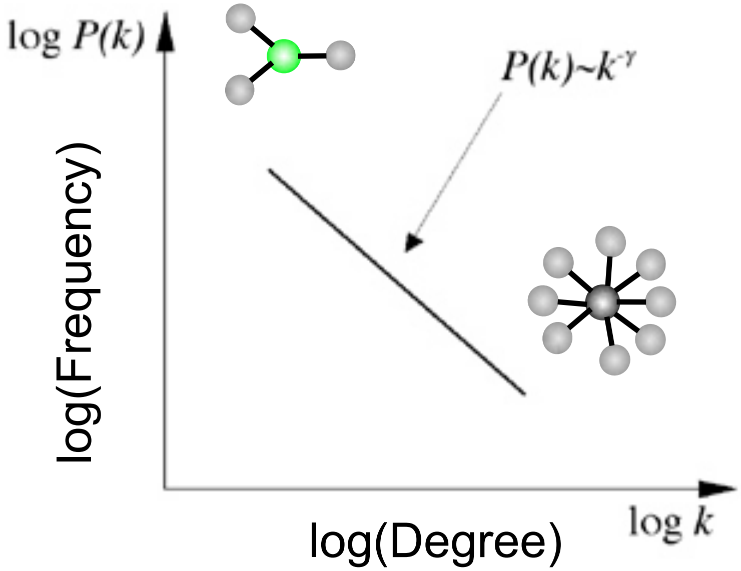
Global topological measures for directed networks

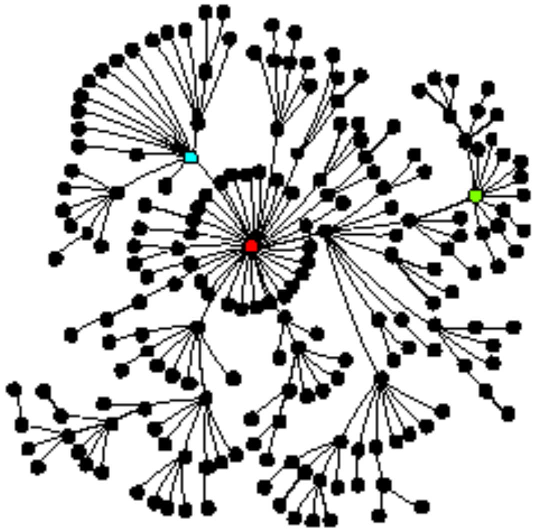


Regulatory and metabolic networks are ***directed***

Scale-free networks

Power-law distribution





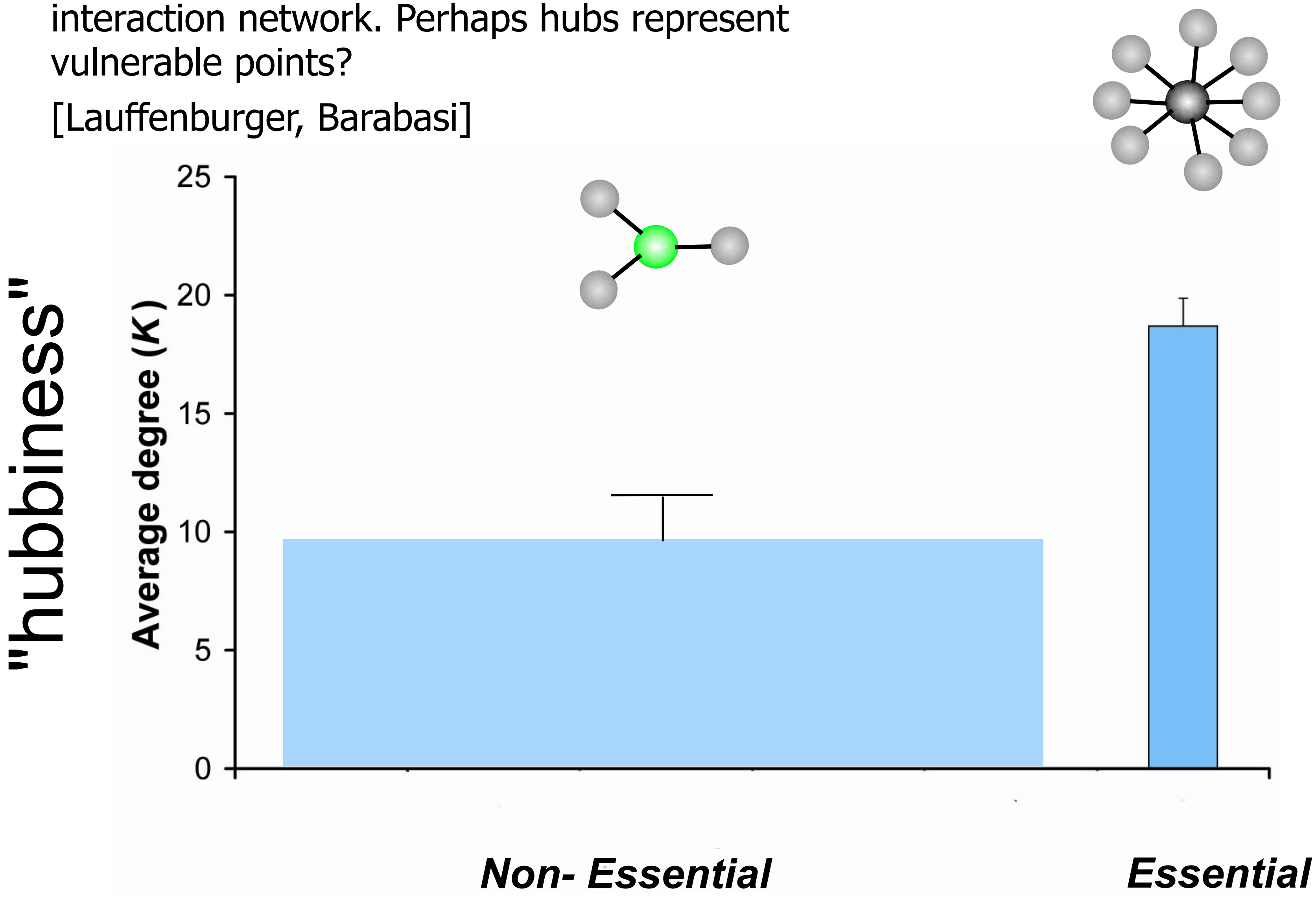
Hubs dictate the structure of the network

[Barabasi]

Hubs tend to be Essential

Integrate gene essentiality data with protein interaction network. Perhaps hubs represent vulnerable points?

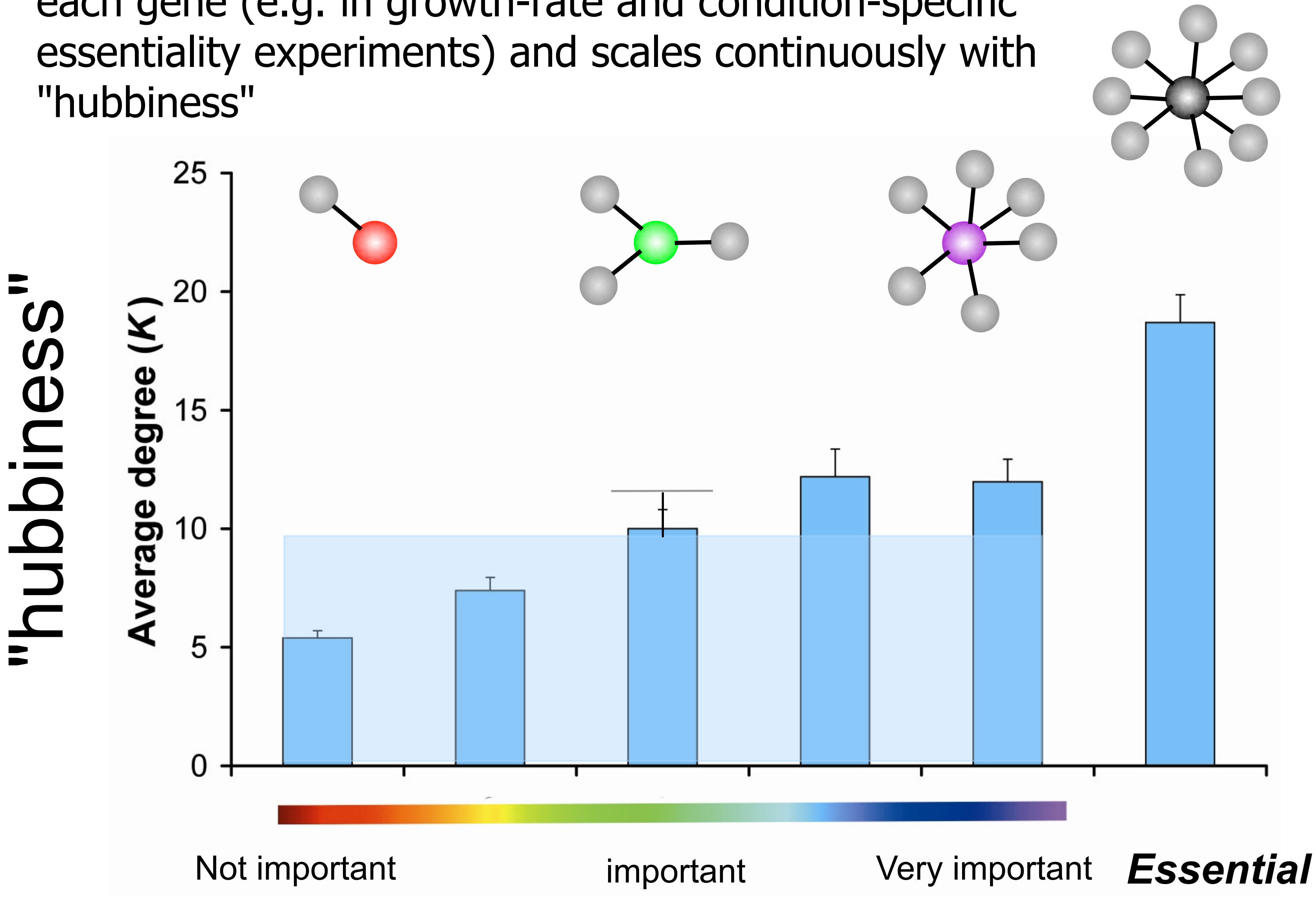
[Lauffenburger, Barabasi]



[Yu et al., 2003, TIG]

Relationships extends to "Marginal Essentiality"

Marginal essentiality measures relative importance of each gene (e.g. in growth-rate and condition-specific essentiality experiments) and scales continuously with "hubbiness"



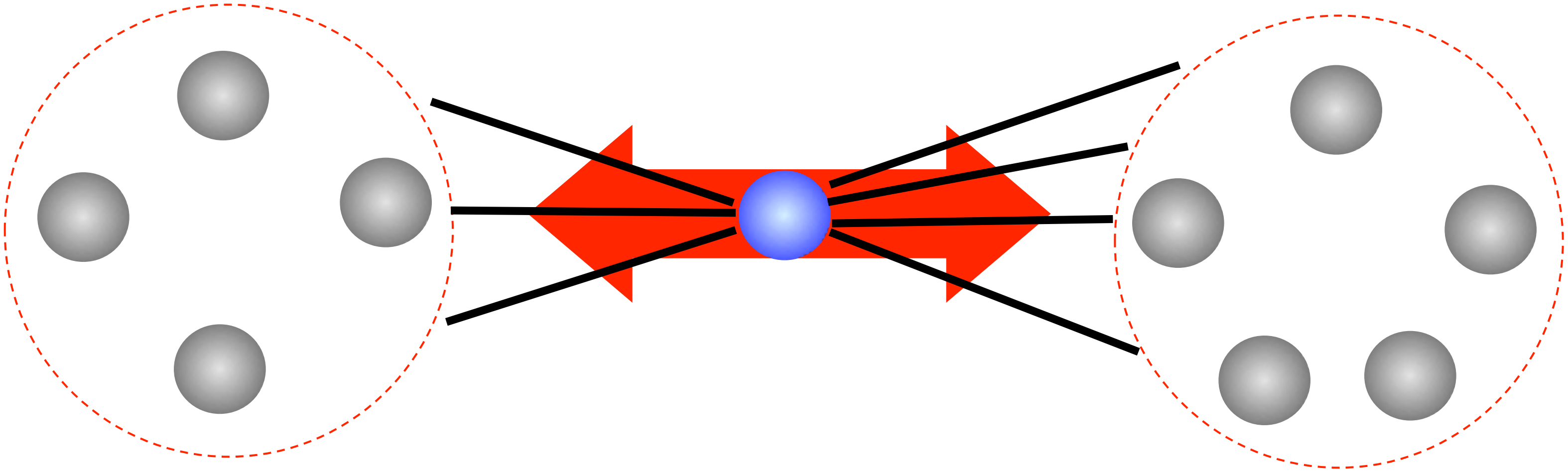
[Yu et al., 2003, TIG]

Another measure of Centrality: Betweenness centrality

Betweenness of a node is the number of shortest paths of pairs of vertices that run through it -- a measure of information flow.

Freeman LC (1977) Set of measures of centrality based on betweenness.
Sociometry 40: 35–41.

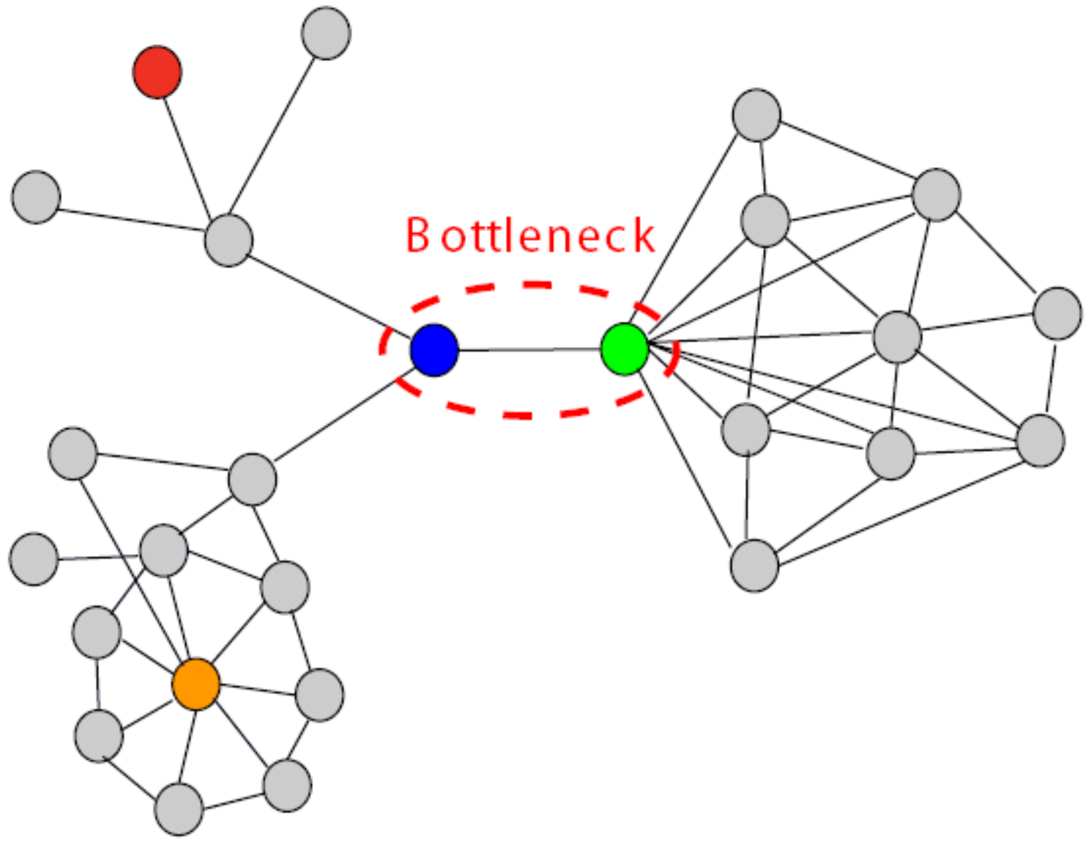
Girvan & Newman (2002) PNAS 99: 7821.



Betweenness centrality -- Bottlenecks

Proteins with high betweenness are defined as *Bottlenecks* (top 20%), in analogy to the traffic system

Bottlenecks & Hubs



🟢 Hub-bottleneck **node**

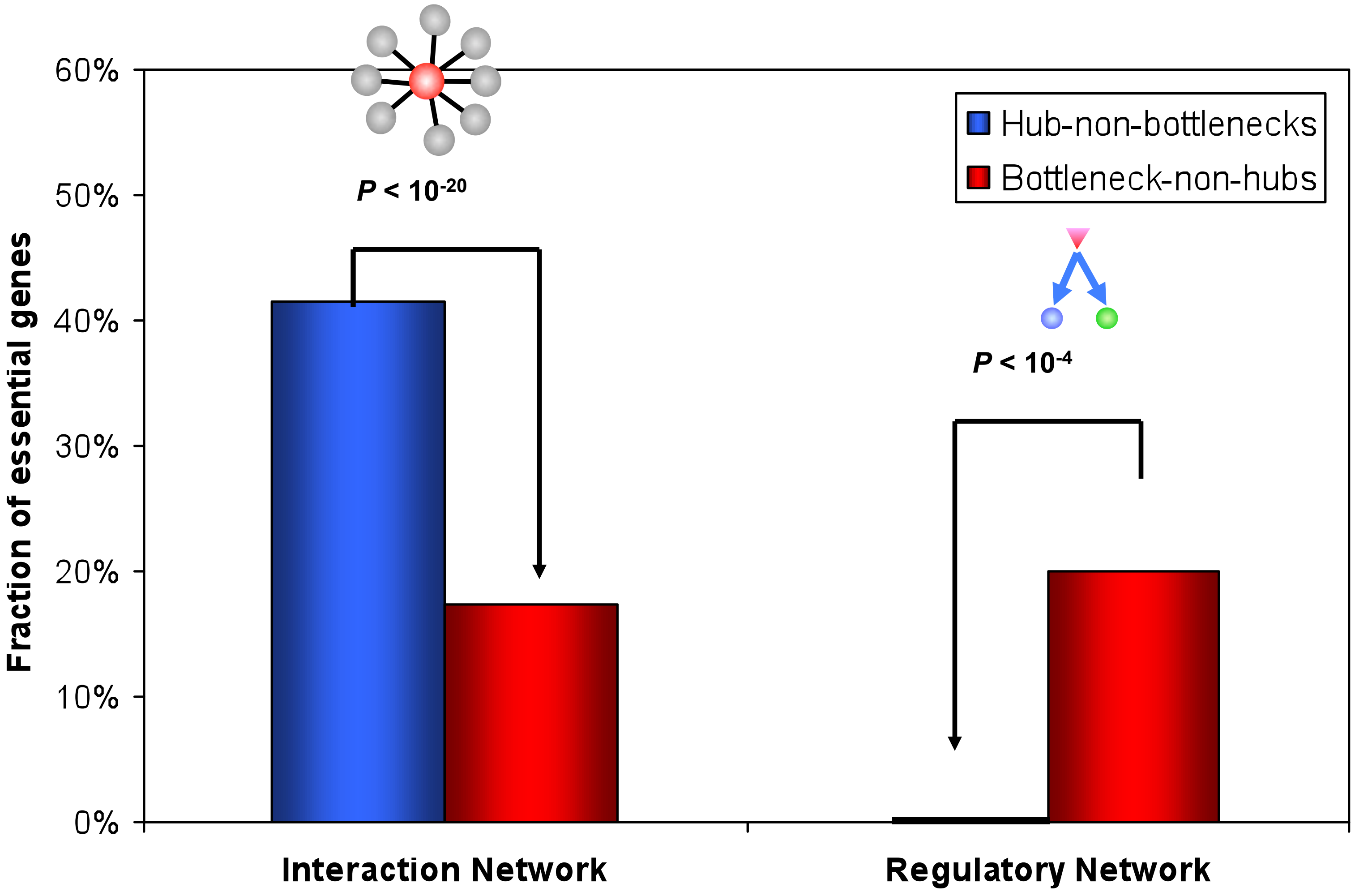
🔵 Non-hub-bottleneck **node**

🟠 Hub-non-bottleneck **node**

🔴 Non-hub-non-bottleneck **node**

[Yu et al., PLOS CB (2007)]

Bottlenecks are what matters in regulatory networks

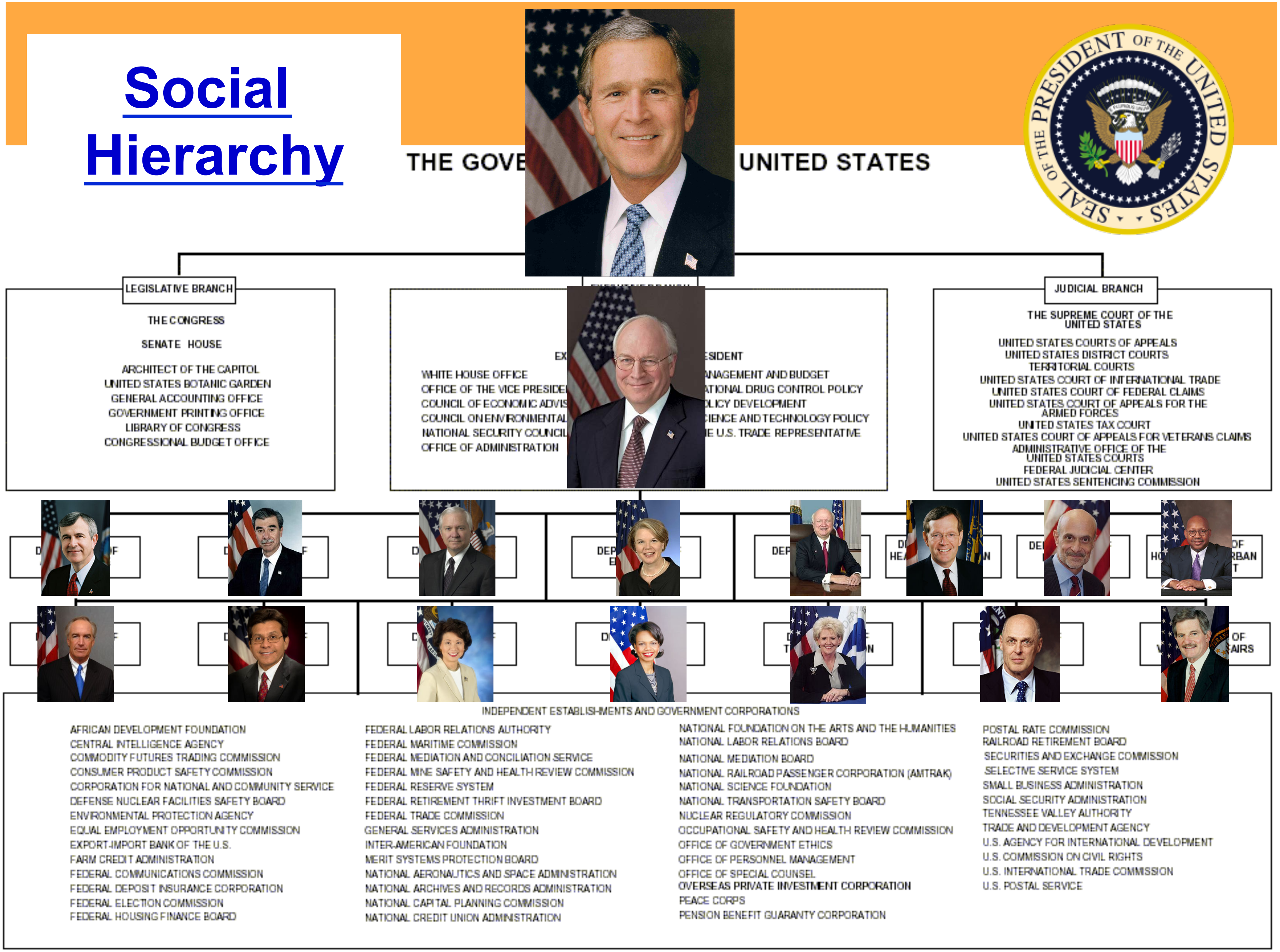


[Yu *et al.*, *PLoS Comput Biol* (2007)]

Finding Central Points in Networks #2: Tops of the Hierarchy

Where are key points networks ? How do we locate them ?

Social Hierarchy



ARCHITECT OF THE CAPITOL
UNITED STATES BOTANIC GARDEN
GENERAL ACCOUNTING OFFICE
GOVERNMENT PRINTING OFFICE
LIBRARY OF CONGRESS
CONGRESSIONAL BUDGET OFFICE

WHITE HOUSE OFFICE
OFFICE OF THE VICE PRESIDE
COUNCIL OF ECONOMIC ADVIS
COUNCIL ON ENVIRONMENTAL
NATIONAL SECURITY COUNCIL
OFFICE OF ADMINISTRATION

ANAGEMENT AND BUDGET
ATIONAL DRUG CONTROL POLICY
OLICY DEVELOPMENT
CIENCE AND TECHNOLOGY POLICY
E U.S. TRADE REPRESENTATIVE

UNITED STATES COURTS OF APPEALS
UNITED STATES DISTRICT COURTS
TERRITORIAL COURTS
UNITED STATES COURT OF INTERNATIONAL TRADE
UNITED STATES COURT OF FEDERAL CLAIMS
UNITED STATES COURT OF APPEALS FOR THE ARMED FORCES
UNITED STATES TAX COURT
UNITED STATES COURT OF APPEALS FOR VETERANS CLAIMS
ADMINISTRATIVE OFFICE OF THE UNITED STATES COURTS
FEDERAL JUDICIAL CENTER
UNITED STATES SENTENCING COMMISSION

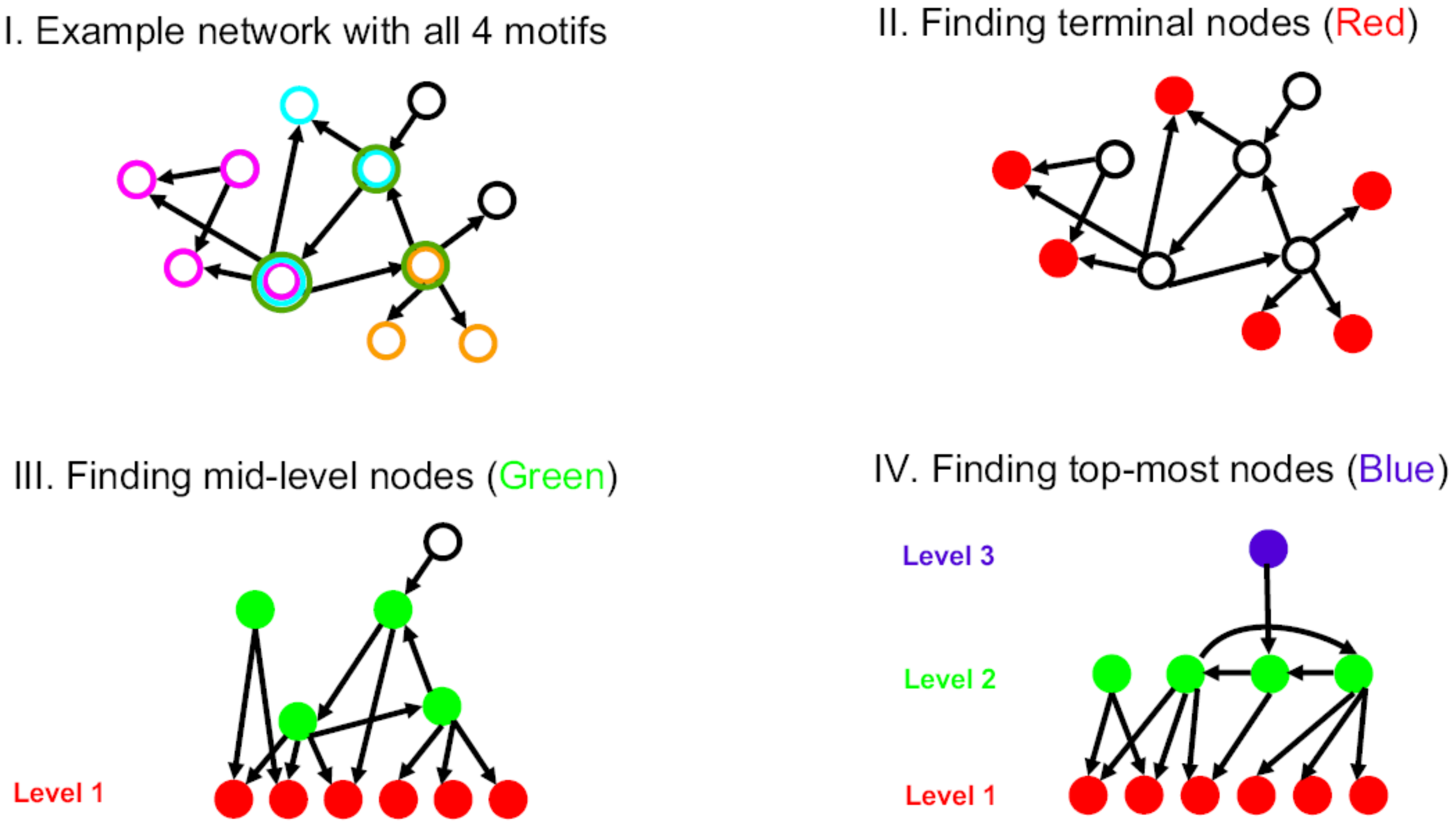
AFRICAN DEVELOPMENT FOUNDATION
CENTRAL INTELLIGENCE AGENCY
COMMODITY FUTURES TRADING COMMISSION
CONSUMER PRODUCT SAFETY COMMISSION
CORPORATION FOR NATIONAL AND COMMUNITY SERVICE
DEFENSE NUCLEAR FACILITIES SAFETY BOARD
ENVIRONMENTAL PROTECTION AGENCY
EQUAL EMPLOYMENT OPPORTUNITY COMMISSION
EXPORT-IMPORT BANK OF THE U.S.
FARM CREDIT ADMINISTRATION
FEDERAL COMMUNICATIONS COMMISSION
FEDERAL DEPOSIT INSURANCE CORPORATION
FEDERAL ELECTION COMMISSION
FEDERAL HOUSING FINANCE BOARD

FEDERAL LABOR RELATIONS AUTHORITY
FEDERAL MARITIME COMMISSION
FEDERAL MEDIATION AND CONCILIATION SERVICE
FEDERAL MINE SAFETY AND HEALTH REVIEW COMMISSION
FEDERAL RESERVE SYSTEM
FEDERAL RETIREMENT THRIFT INVESTMENT BOARD
FEDERAL TRADE COMMISSION
GENERAL SERVICES ADMINISTRATION
INTER-AMERICAN FOUNDATION
MERIT SYSTEMS PROTECTION BOARD
NATIONAL AERONAUTICS AND SPACE ADMINISTRATION
NATIONAL ARCHIVES AND RECORDS ADMINISTRATION
NATIONAL CAPITAL PLANNING COMMISSION
NATIONAL CREDIT UNION ADMINISTRATION

NATIONAL FOUNDATION ON THE ARTS AND THE HUMANITIES
NATIONAL LABOR RELATIONS BOARD
NATIONAL MEDIATION BOARD
NATIONAL RAILROAD PASSENGER CORPORATION (AMTRAK)
NATIONAL SCIENCE FOUNDATION
NATIONAL TRANSPORTATION SAFETY BOARD
NUCLEAR REGULATORY COMMISSION
OCCUPATIONAL SAFETY AND HEALTH REVIEW COMMISSION
OFFICE OF GOVERNMENT ETHICS
OFFICE OF PERSONNEL MANAGEMENT
OFFICE OF SPECIAL COUNSEL
OVERSEAS PRIVATE INVESTMENT CORPORATION
PEACE CORPS
PENSION BENEFIT GUARANTY CORPORATION

POSTAL RATE COMMISSION
RAILROAD RETIREMENT BOARD
SECURITIES AND EXCHANGE COMMISSION
SELECTIVE SERVICE SYSTEM
SMALL BUSINESS ADMINISTRATION
SOCIAL SECURITY ADMINISTRATION
TENNESSEE VALLEY AUTHORITY
TRADE AND DEVELOPMENT AGENCY
U.S. AGENCY FOR INTERNATIONAL DEVELOPMENT
U.S. COMMISSION ON CIVIL RIGHTS
U.S. INTERNATIONAL TRADE COMMISSION
U.S. POSTAL SERVICE

Determination of "Level" in Regulatory Network Hierarchy with Breadth-first Search



I. Example network with all 4 motifs

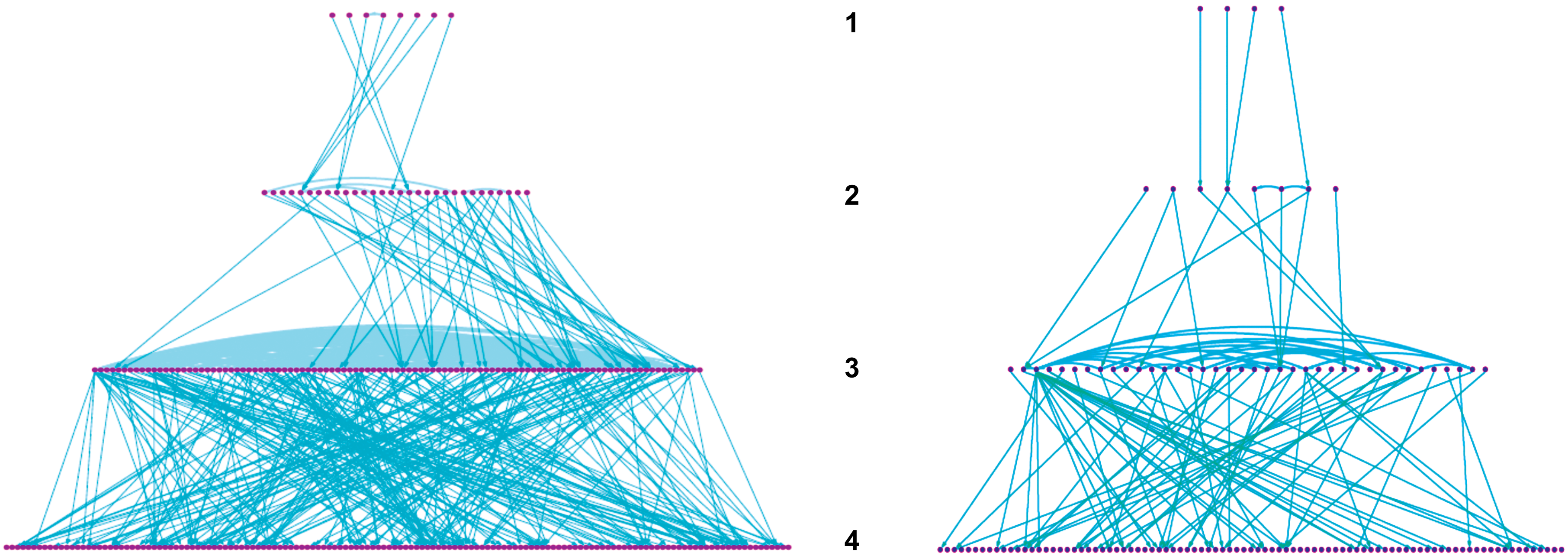
II. Finding terminal nodes (Red)

III. Finding mid-level nodes (Green)

IV. Finding top-most nodes (Blue)

[Yu et al., PNAS (2006)]

Regulatory Networks have similar hierarchical structures

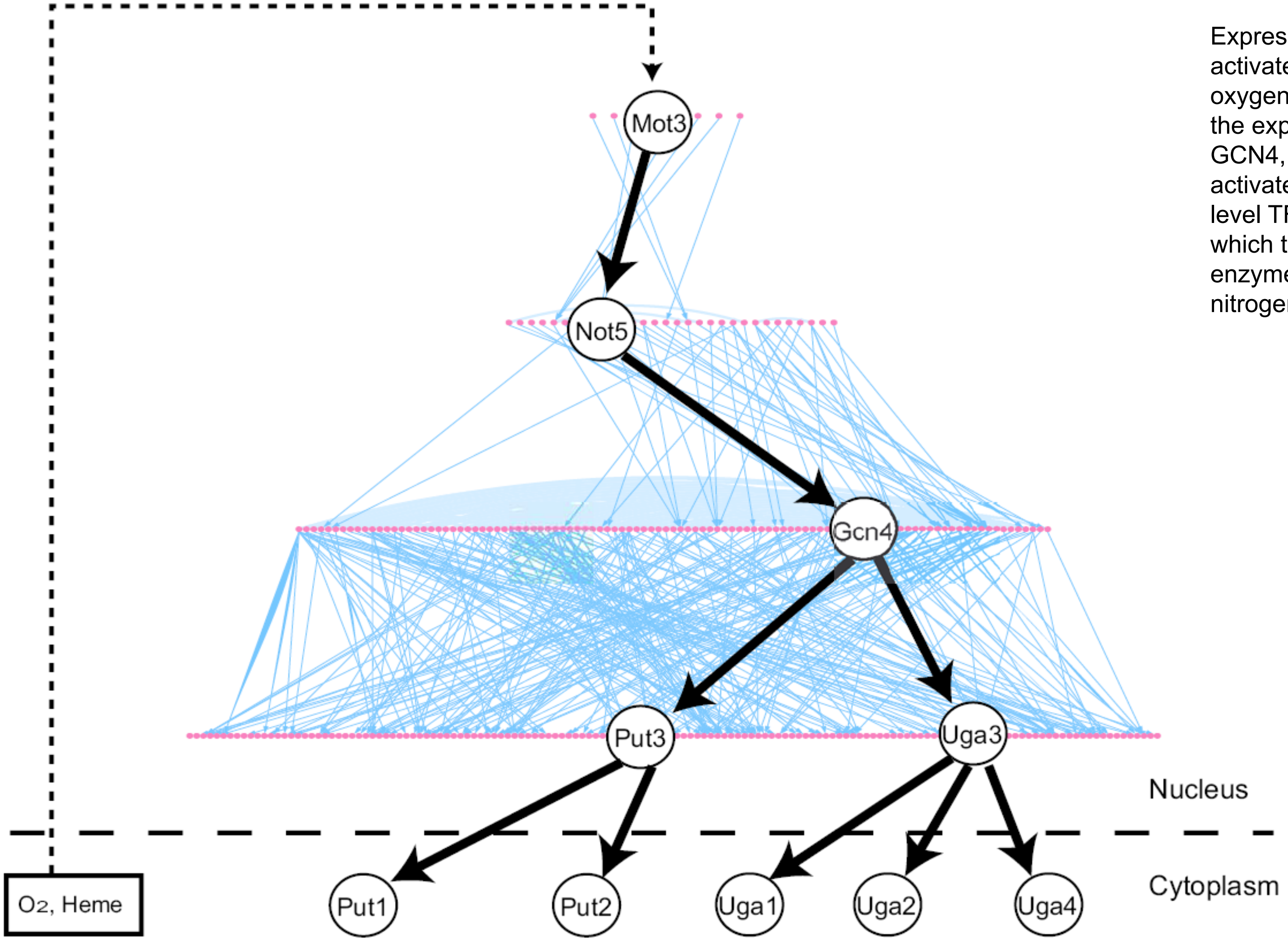


S. cerevisiae

E. coli

[Yu *et al.*, *Proc Natl Acad Sci U S A* (2006)]

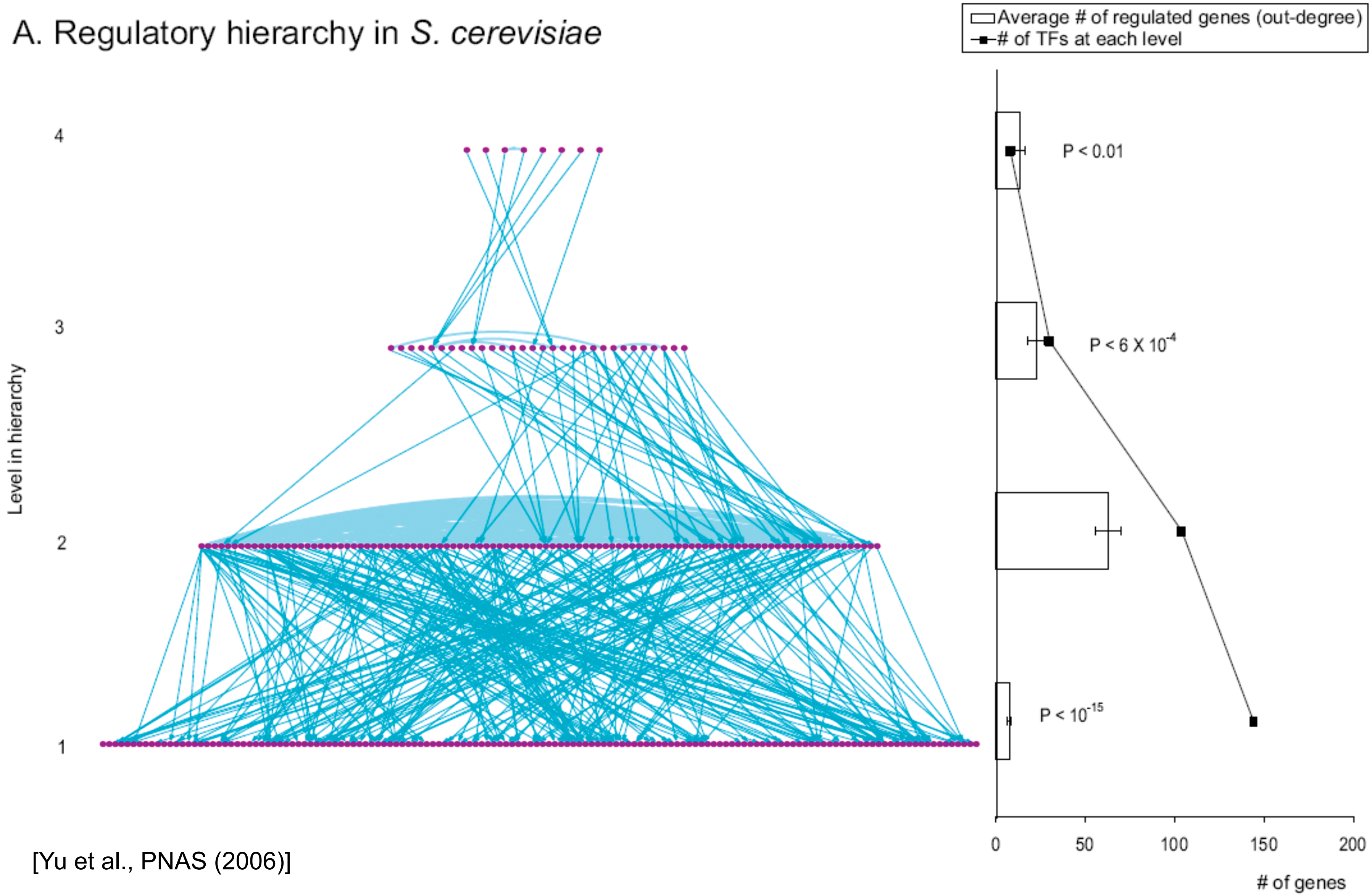
Example of Path Through Regulatory Network



Expression of MOT3 is activated by heme and oxygen. Mot3 in turn activates the expression of NOT5 and GCN4, mid-level hubs. GCN4 activates two specific bottom-level TFs, Put3 and Uga3, which trigger the expression of enzymes in proline and nitrogen utilization.

[Yu et al., PNAS (2006)]

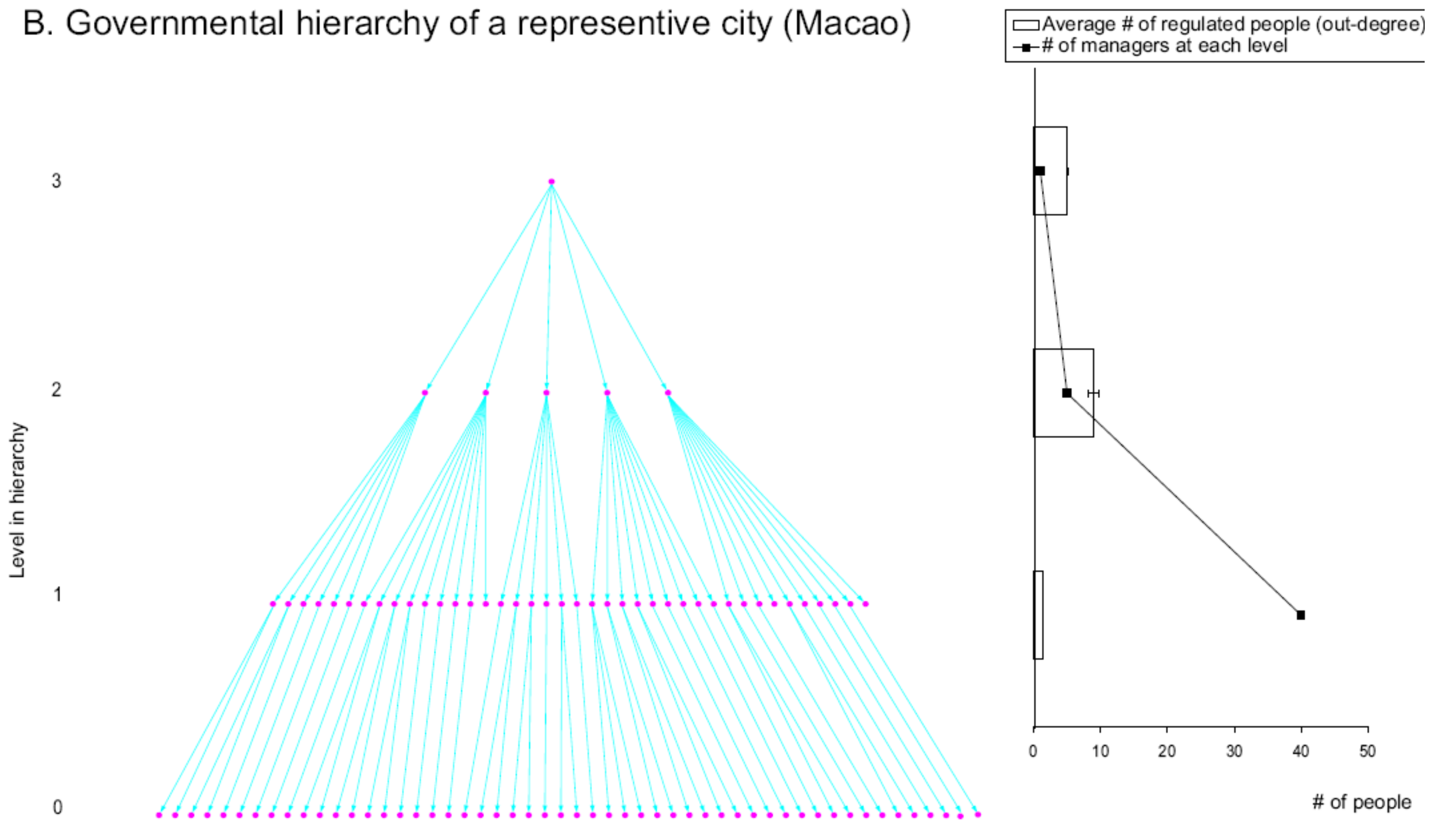
Yeast Regulatory Hierarchy: the Middle-managers Rule



A. Regulatory hierarchy in *S. cerevisiae*

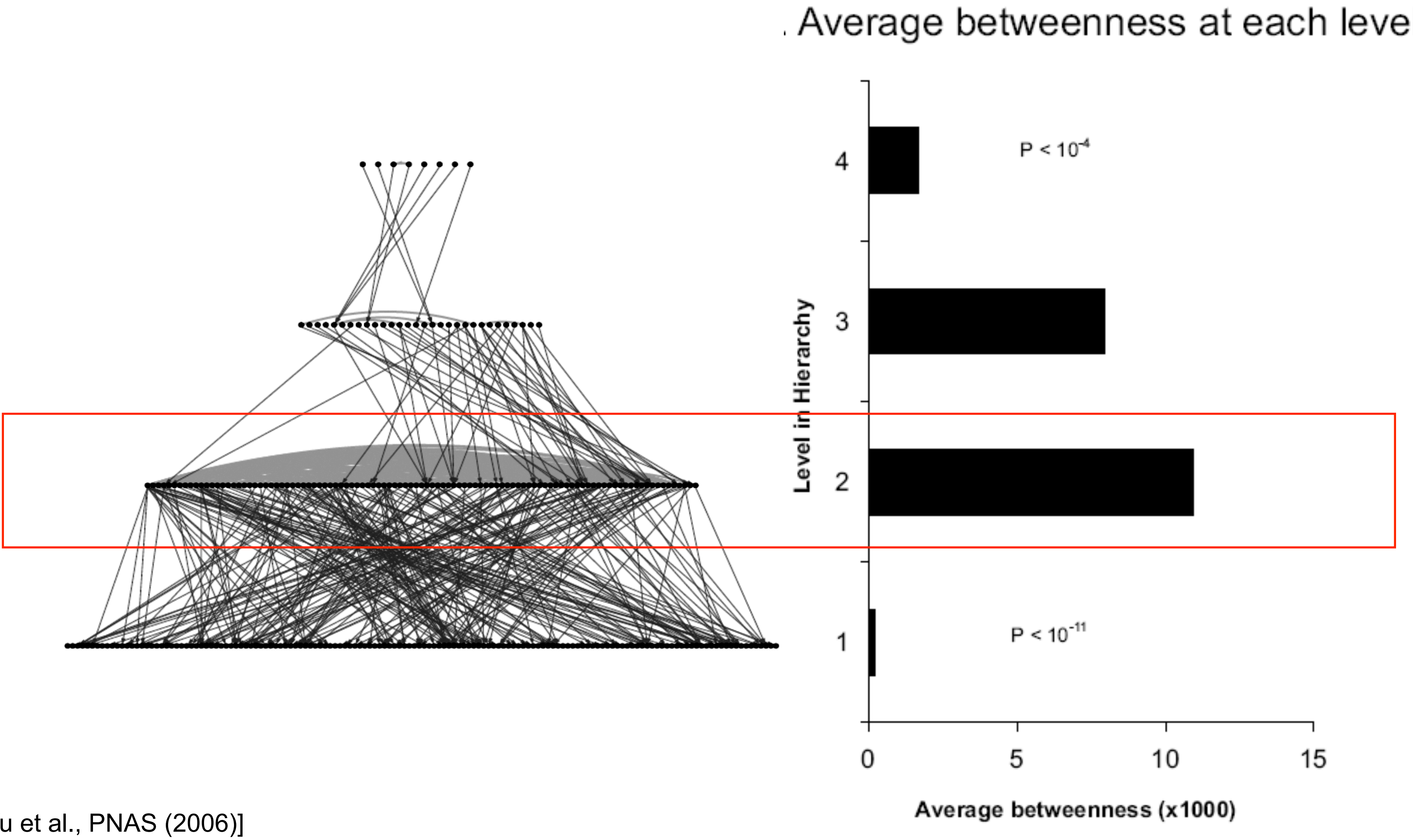
[Yu et al., PNAS (2006)]

Yeast Network Similar in Structure to Government Hierarchy with Respect to Middle-managers



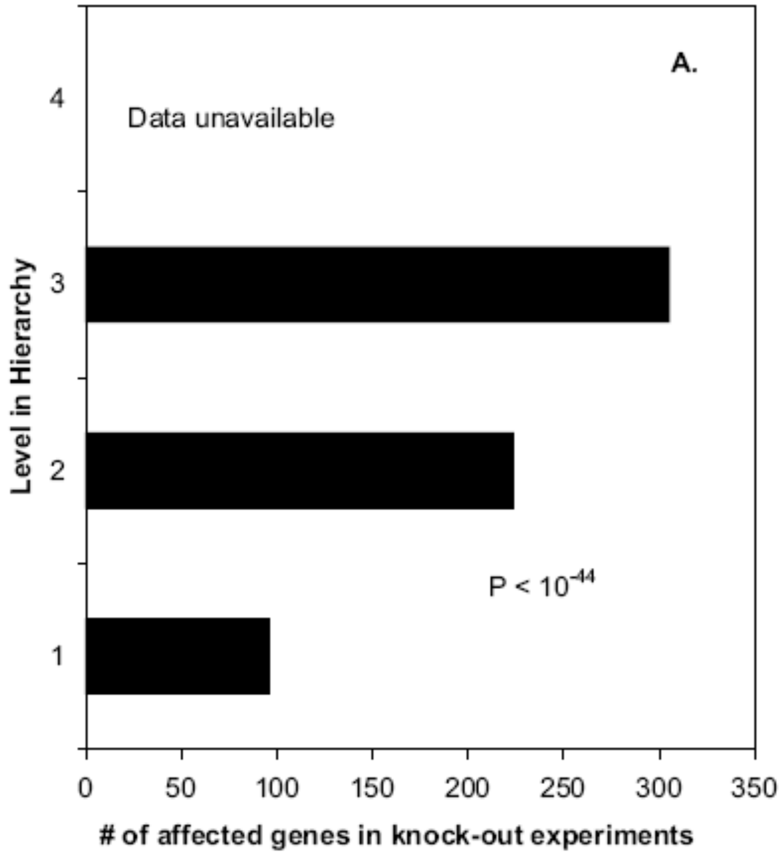
B. Governmental hierarchy of a representive city (Macao)

Characteristics of Regulatory Hierarchy: Middle Managers are Information Flow Bottlenecks



[Yu et al., PNAS (2006)]

Characteristics of Regulatory Hierarchy: The Paradox of Influence and Essentiality

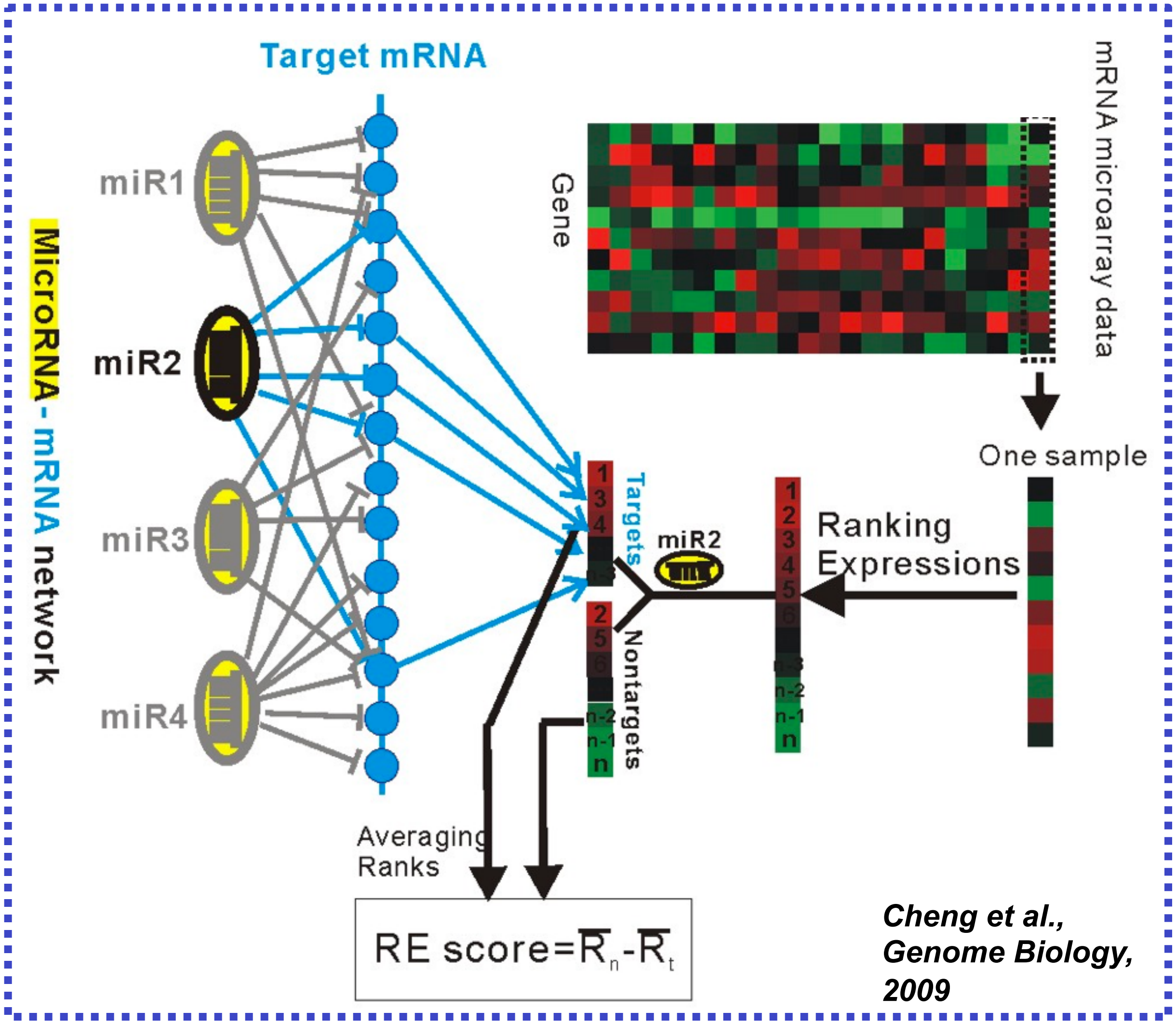


[Yu et al., PNAS (2006)]

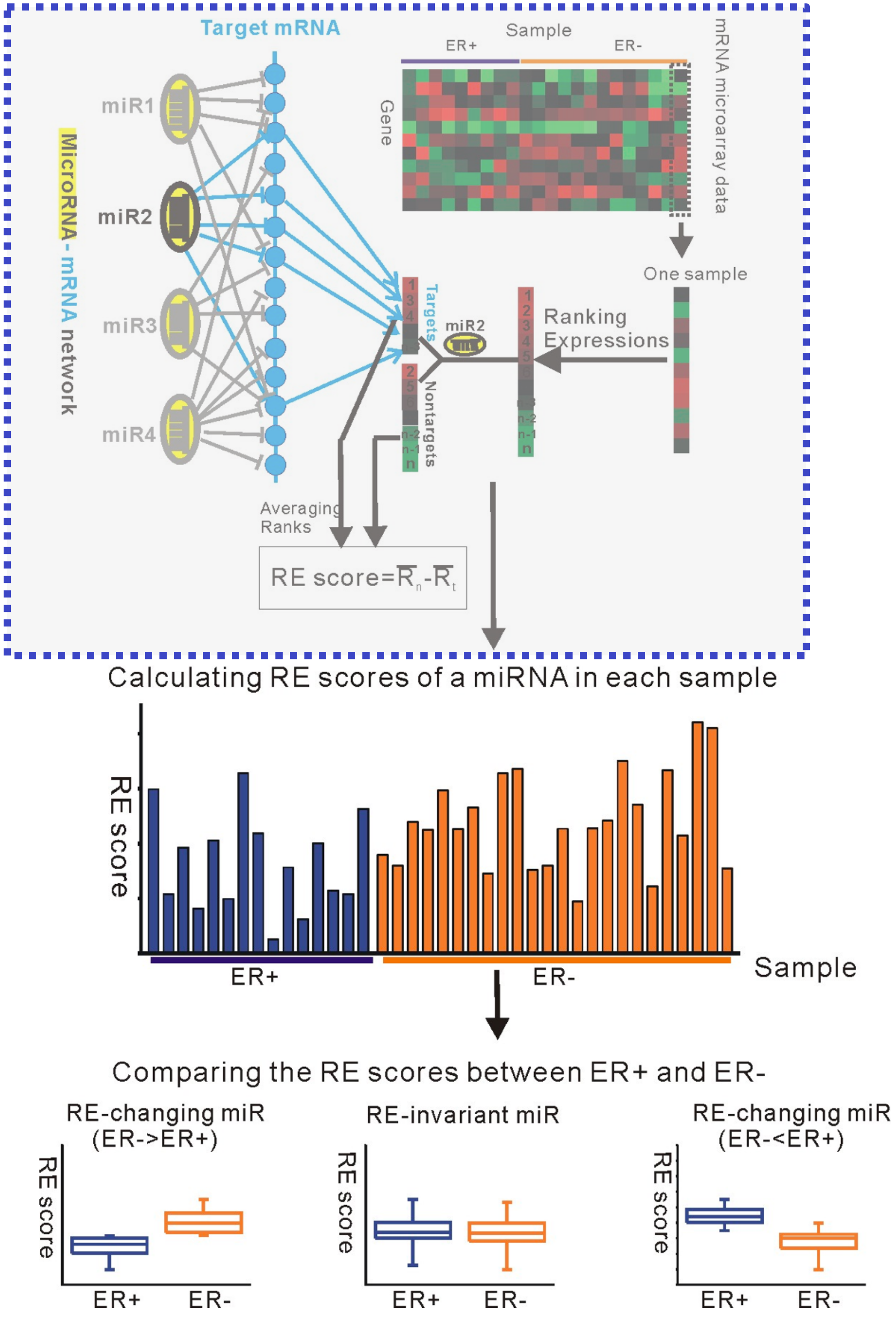
Finding Central Points in Networks #3: Points of Maximal Regulatory Effect

RE-score: Another way to identify "important" network nodes

- How much does a regulator influence its targets?
- For miRNA-target networks easy to calculate, as all influence is down-regulation
    - ◊ target prediction via: TargetScan, PITA, PicTar, miRanda, …
- Look at down-reg. genes in a sample & compare with targets of a specific micro-RNA
    - ◊ more down-reg genes => stronger regulatory effect



Cheng et al., Genome Biology, 2009



Application of RE-score to measure changing miRNA effect in different conditions

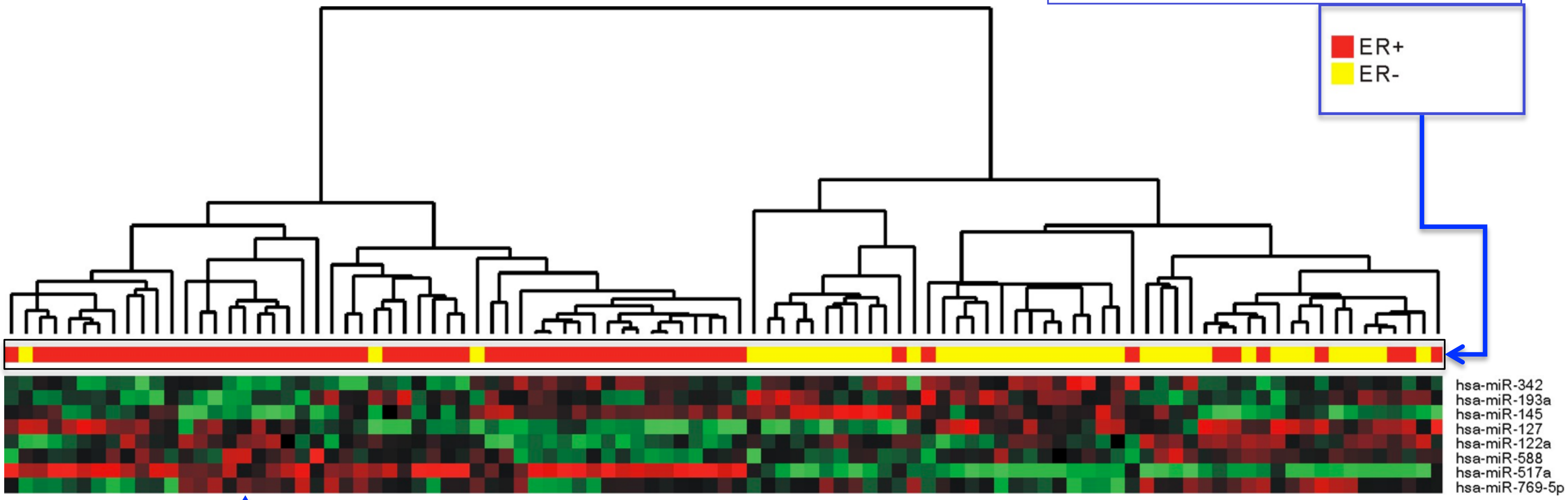
(ER- and ER+ breast cancer)

Cheng et al., Genome Biology, 2009

RE-score can be used to classify cancers

(3) Clustering based on RE score divides samples into 2 main types of cancer

(4) Clustering better than based on indiv. gene expression levels

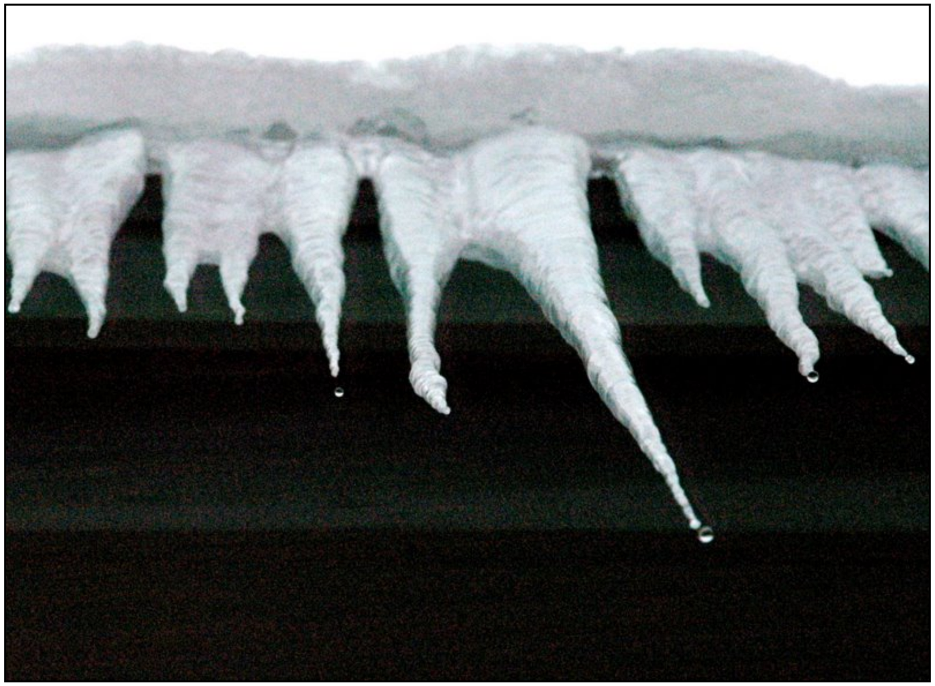


(1) RE-score profile for diff. miRNA in 1 cancer sample.
(2) Tabulate over many different breast cancer samples

Cheng et al., Genome Biology, 2009

Network Dynamics #2: Environments

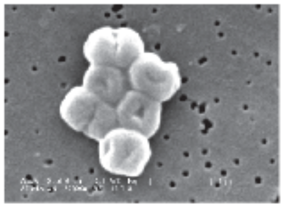
How do molecular networks change across environments?
What pathways are used more ?
Used as a biosensor ?



What is metagenomics?

Genomics Approach

Culture Microbes

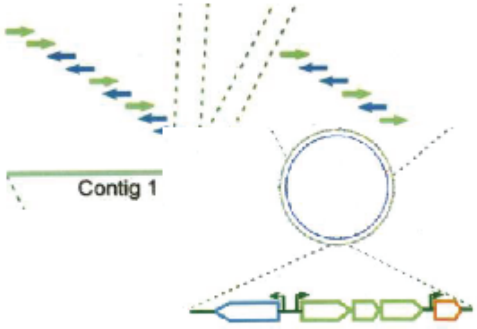


Extract DNA

Sequence

ATCGTATA
CGCGAAG
ACGTCTGA
AGTGCTGCT

Assemble and Annotate



PROBLEM: Estimated that less than 1% can be cultured in the lab

Metagenomics Approach

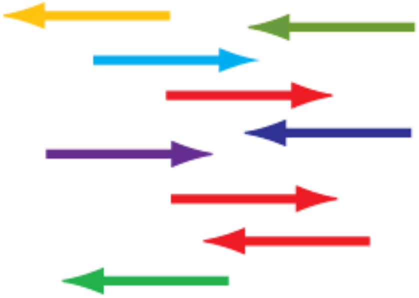
Collect Sample

Extract DNA

Sequence

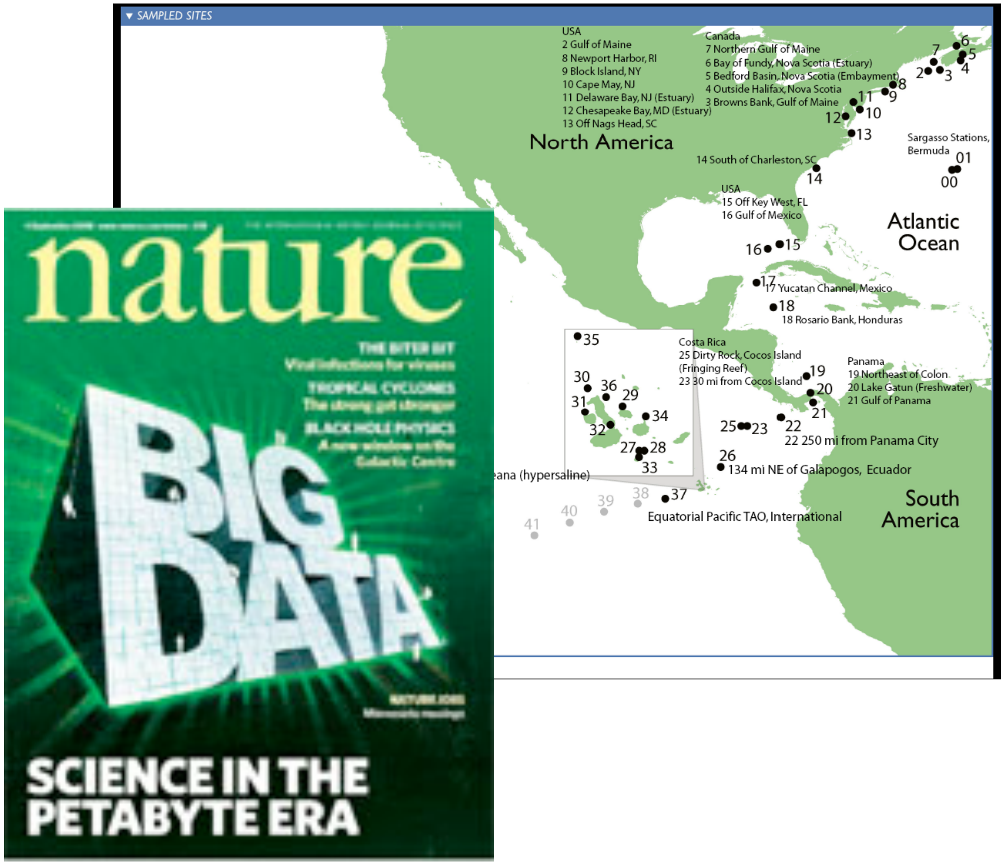
ATCGTGATAGATGATAGTAGA
ATGCTGCATGCATCTAGCACT
ACAGTAGCTAGCTACGTACTA
CAGCTGACTAGCTAGCTAGCT
ACGTAGCATGCTAGCTAGCAG
ACGTACGTAGCTAGCTAGCTAG
ACGTACGTACGTAGCTAGCATC
AGTCGACTGAGCCAGTGATGAT
ACGATGCATGAGCAGATGCTAC
AGATCGTAGCATGCTAGCATGCT
ACGTACGTAGCTAGCTAGCTAAG
AGCTAGCATGCTAGTAGCATGAG
ACGATGCTAGCTAGCTAGCTGATA
TCGATCAGCATGCTACGATGCAAG
ACGATCGATGCTAGCTAGCTAGCAT
AGCTAGCTAGTCAGCTAGCTAGATG

Partially Assemble and Annotate



PROBLEM: Lose information about which gene belongs to which microbe.

Global Ocean Survey Statistics (GOS)

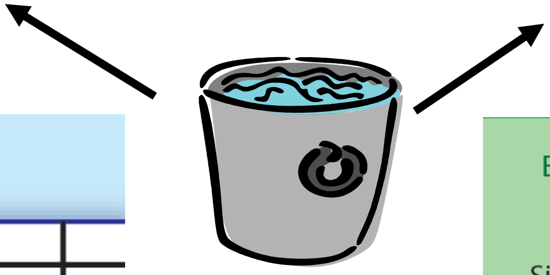


SAMPLED SITES
USA
2 Gulf of Maine
8 Newport Harbor, RI
9 Block Island, NY
10 Cape May, NJ
11 Delaware Bay, NJ (Estuary)
12 Chesapeake Bay, MD (Estuary)
13 Off Nags Head, SC
Canada
7 Northern Gulf of Maine
6 Bay of Fundy, Nova Scotia (Estuary)
5 Bedford Basin, Nova Scotia (Embayment)
4 Outside Halifax, Nova Scotia
3 Browns Bank, Gulf of Maine
North America
14 South of Charleston, SC
Sargasso Stations, Bermuda
01
00
USA
15 Off Key West, FL
16 Gulf of Mexico
Atlantic Ocean
17 Yucatan Channel, Mexico
18 Rosario Bank, Honduras
Costa Rica
25 Dirty Rock, Cocos Island (Fringing Reef)
23 30 mi from Cocos Island
Panama
19 Northeast of Colon
20 Lake Gatun (Freshwater)
21 Gulf of Panama
22 250 mi from Panama City
26 134 mi NE of Galapogos, Ecuador
ana (hypersaline)
Equatorial Pacific TAO, International
South America
nature
THE BITER BIT
Viral infections for viruses
TROPICAL CYCLONES
The strong get stronger
BLACK HOLE PHYSICS
A new window on the Galactic Centre
BIG DATA
SCIENCE IN THE PETABYTE ERA

6.25 GB of data
7.7M Reads
1 million CPU hours to process

Rusch, et al., PLOS Biology 2007

Pathway Sequences
(Community Function)

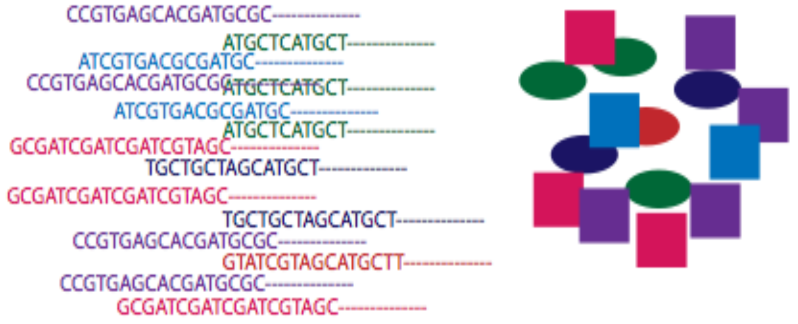


Environmental
Features

	Metabolic Pathways	P1	P2	P3		
Sites	B1	3800	1400	1000		
	B2	2200	100	400		
	↓	----	----	----		

	Environmental Metadata	Temp	NaCl	Depth		
Sites	B1	15˚C	27.2	10 m		
	B2	23˚C	36.6	5 m		
	↓	----	---	-----		

READS → PROTEIN FAMILIES → PATHWAYS


CCGTGAGCACGATGCGC--------------
ATGCTCATGCT--------------
ATCGTGACGCGATGC--------------
CCGTGAGCACGATGCGC
ATGCTCATGCT--------------
ATCGTGACGCGATGC--------------
ATGCTCATGCT--------------
GCGATCGATCGATCGTAGC--------------
TGCTGCTAGCATGCT--------------
GCGATCGATCGATCGTAGC--------------
TGCTGCTAGCATGCT--------------
CCGTGAGCACGATGCGC--------------
GTATCGTAGCATGCTT--------------
CCGTGAGCACGATGCGC--------------
GCGATCGATCGATCGTAGC--------------

$P_1 = f_1 + f_2 + f_3$

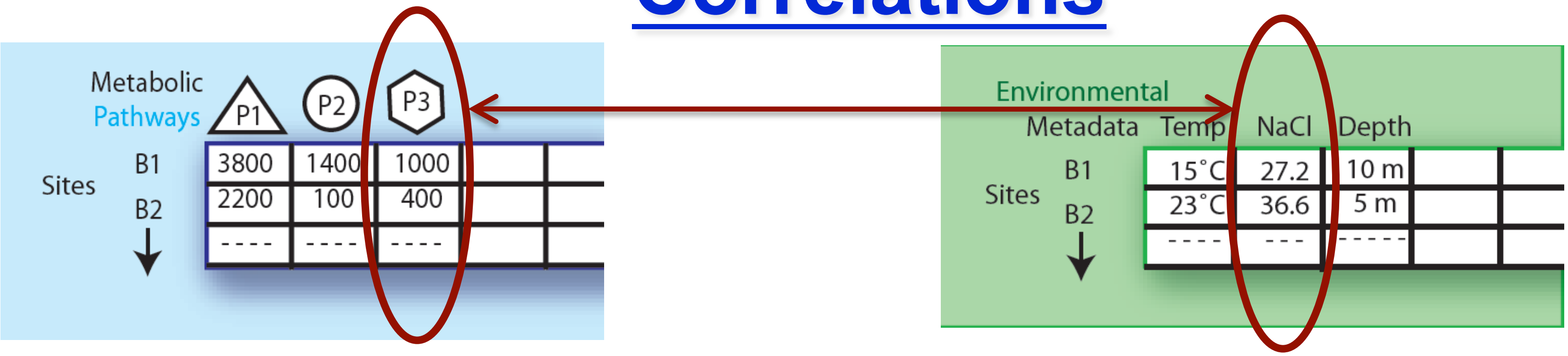
$P_2 = f_4 + f_5 + f_6$

	PATHWAYS	
	●	■
SITES	$P_{1,1} = 2 + 1 + 3$	$P_{2,1} = 2 + 4 + 3$
	$P_{1,2} = 5 + 2 + 6$	$P_{2,1} = 5 + 7 + 6$

Expressing data as matrices indexed by site, env. var., and pathway usage

[Rusch et. al., (2007) PLOS Biology;
Gianoulis et al., PNAS (in press, 2009]

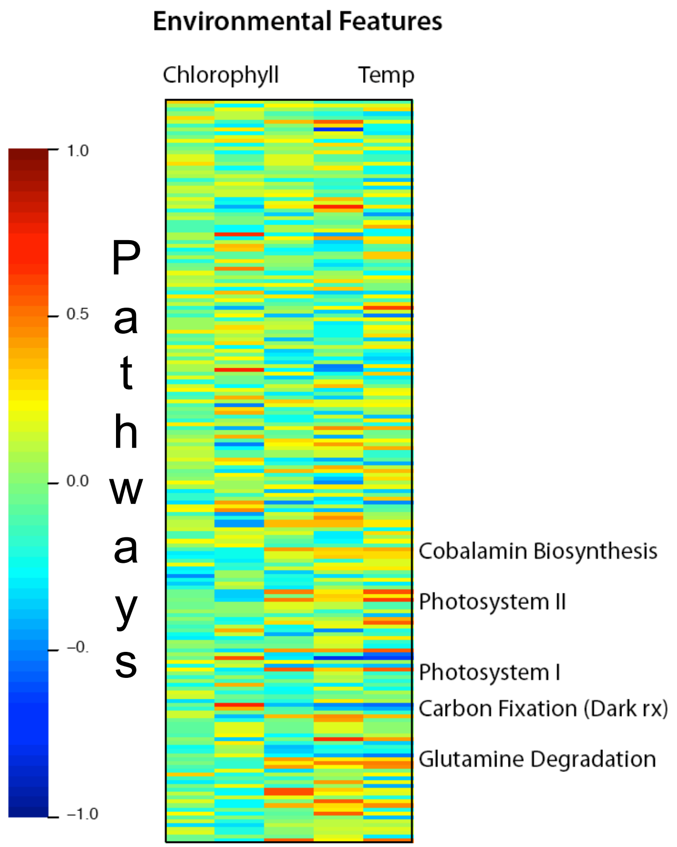
Simple Relationships: Pairwise Correlations

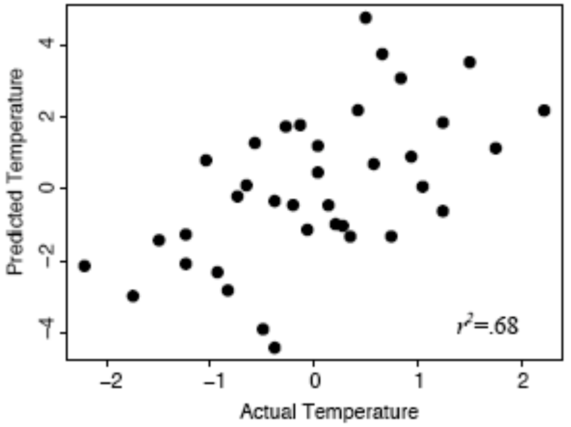


Metabolic Pathways		P1	P2	P3		
Sites	B1	3800	1400	1000		
	B2	2200	100	400		
	↓	----	----	----		

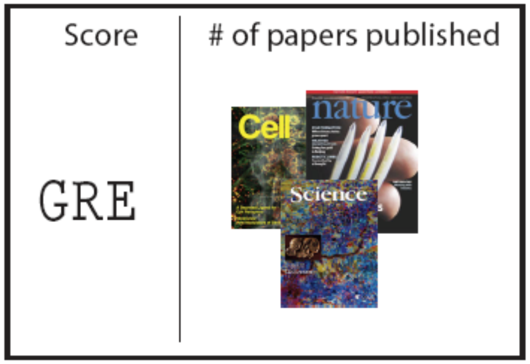
Environmental Metadata		Temp	NaCl	Depth		
Sites	B1	15˚C	27.2	10 m		
	B2	23˚C	36.6	5 m		
	↓	----	---	-----		

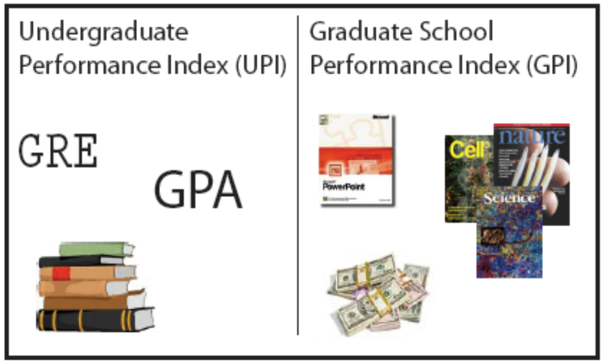
[Gianoulis et al., PNAS (in press, 2009)]

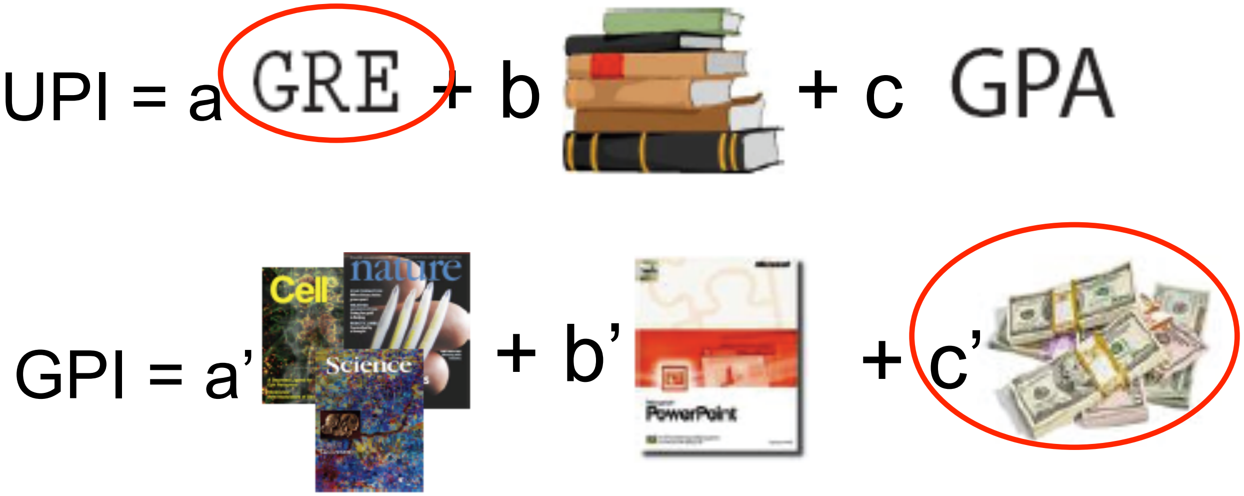




Canonical Correlation Analysis: Simultaneous weighting

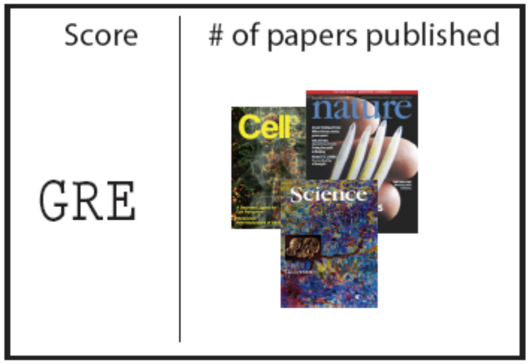


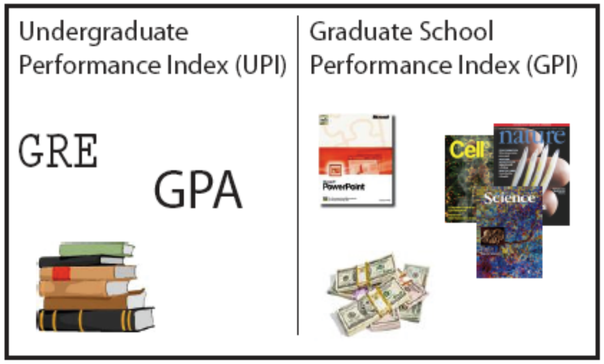

Undergraduate Performance Index (UPI)
Graduate School Performance Index (GPI)
GRE
GPA
PowerPoint
Cell
nature
Science

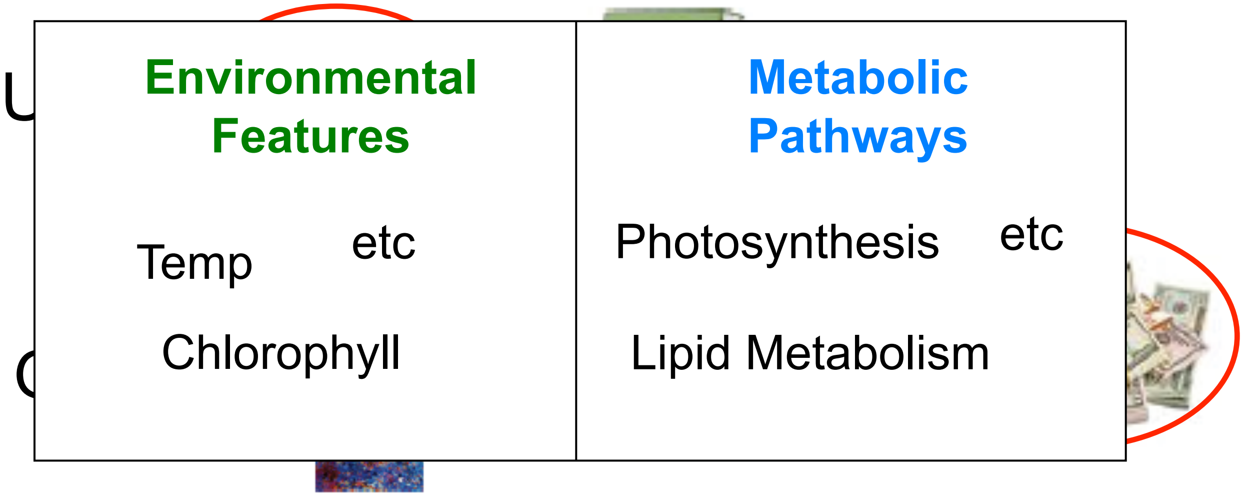


[Gianoulis et al., PNAS (in press, 2009)]

Canonical Correlation Analysis: Simultaneous weighting




Undergraduate Performance Index (UPI)
Graduate School Performance Index (GPI)
GRE
GPA
PowerPoint
Cell
nature
Science



Environmental Features

Temp

etc

Chlorophyll

Metabolic Pathways

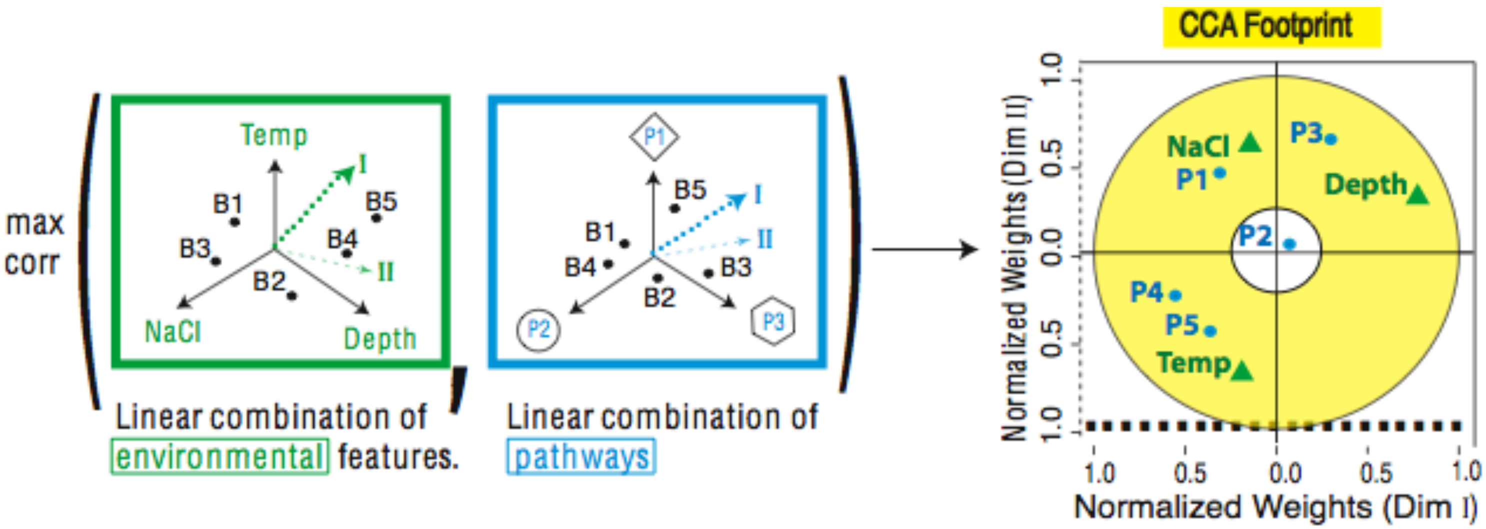
Photosynthesis

etc

Lipid Metabolism

[Gianoulis et al., PNAS (in press, 2009)]

Environmental-Metabolic Space

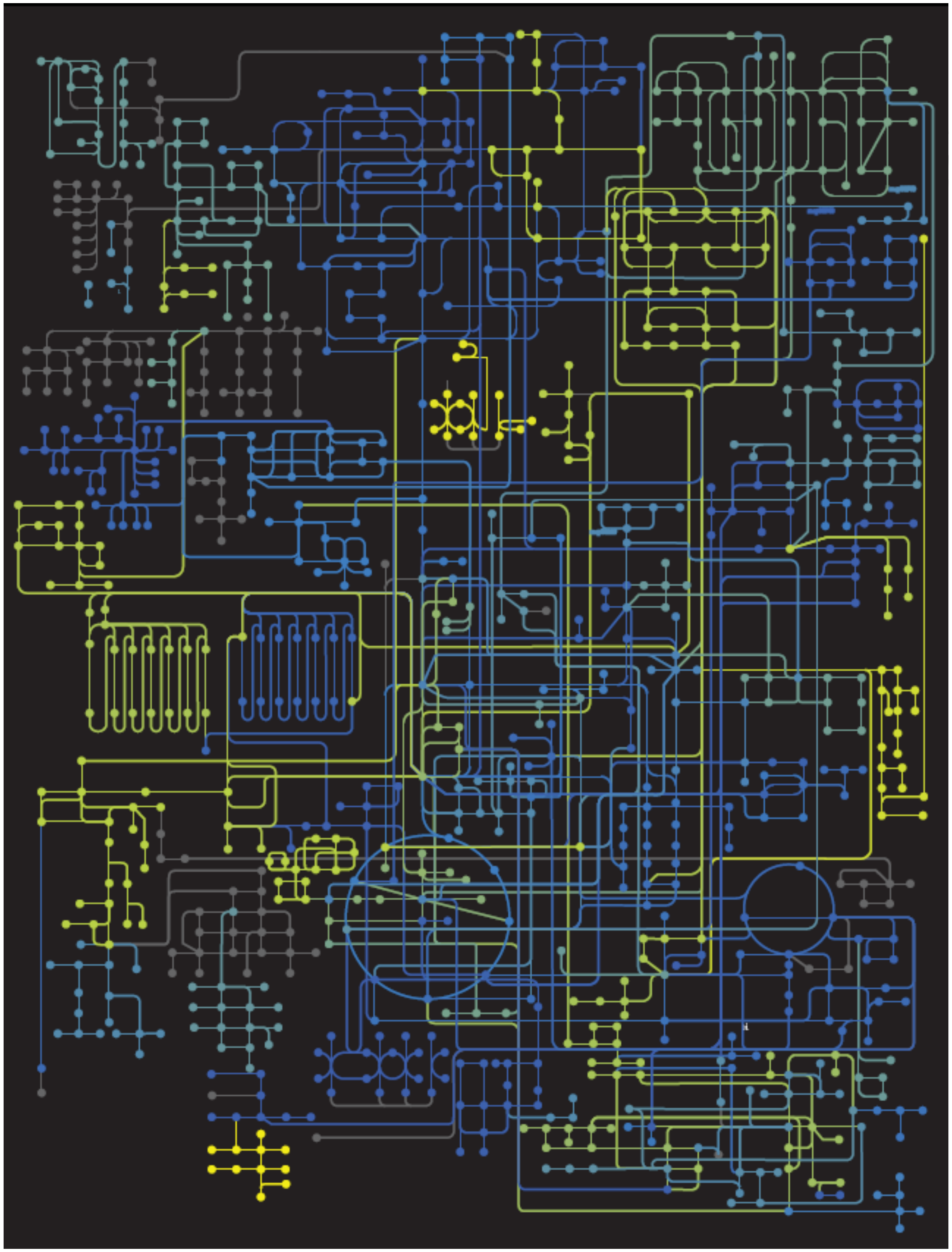

max corr
Temp
I
B1
B5
B3
B4
II
B2
NaCl
Depth
Linear combination of environmental features.
P1
B5
I
B1
II
B4
B3
B2
P2
P3
Linear combination of pathways
CCA Footprint
Normalized Weights (Dim II)
1.0
0.5
0.0
0.5
1.0
NaCl
P1
P3
Depth
P2
P4
P5
Temp
1.0
0.5
0.0
0.5
1.0
Normalized Weights (Dim I)

The goal of this technique is to interpret cross-variance matrices
We do this by defining a change of basis.

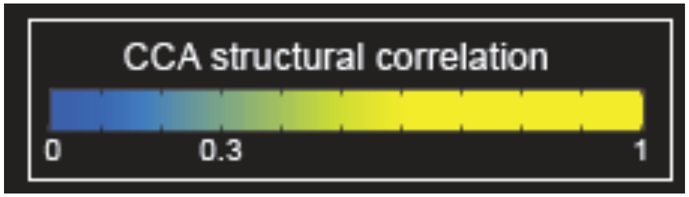
$$\text{Given } X = \{x_1, x_2, \ldots, x_n\} \text{ and } Y = \{y_1, y_2, \ldots, y_m\}$$

$$C = \begin{matrix} \Sigma_X & \Sigma_{X,Y} \\ \Sigma_Y & \Sigma_{Y,X} \end{matrix} \qquad \max_{a,b} Corr(U,V) = \frac{a' \Sigma_{12} b}{\sqrt{a' \Sigma_{11} a} \sqrt{b' \Sigma_{22} b}}$$

[Gianoulis et al., PNAS (in press, 2009)]

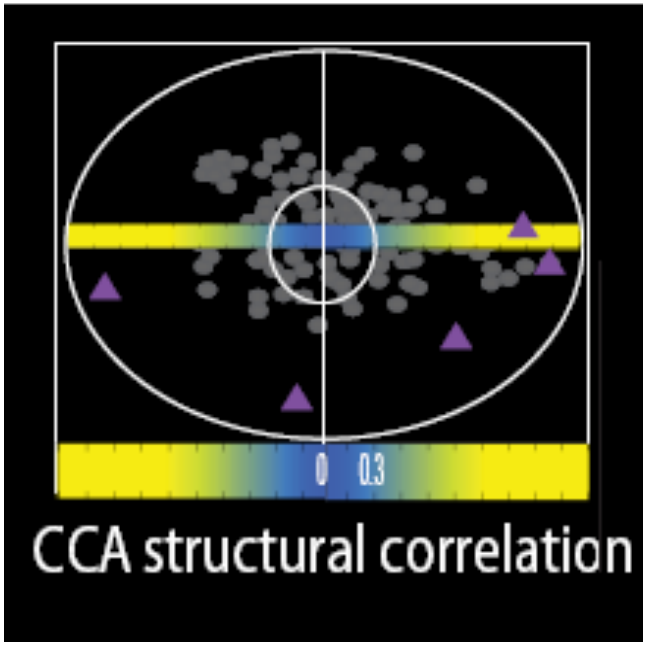


Strength of Pathway co-variation with environment



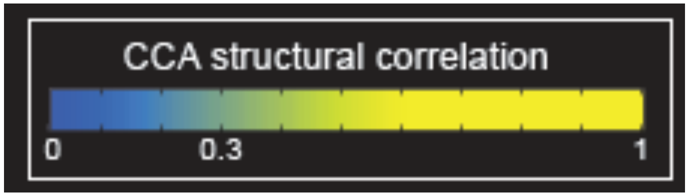
Environmentally invariant

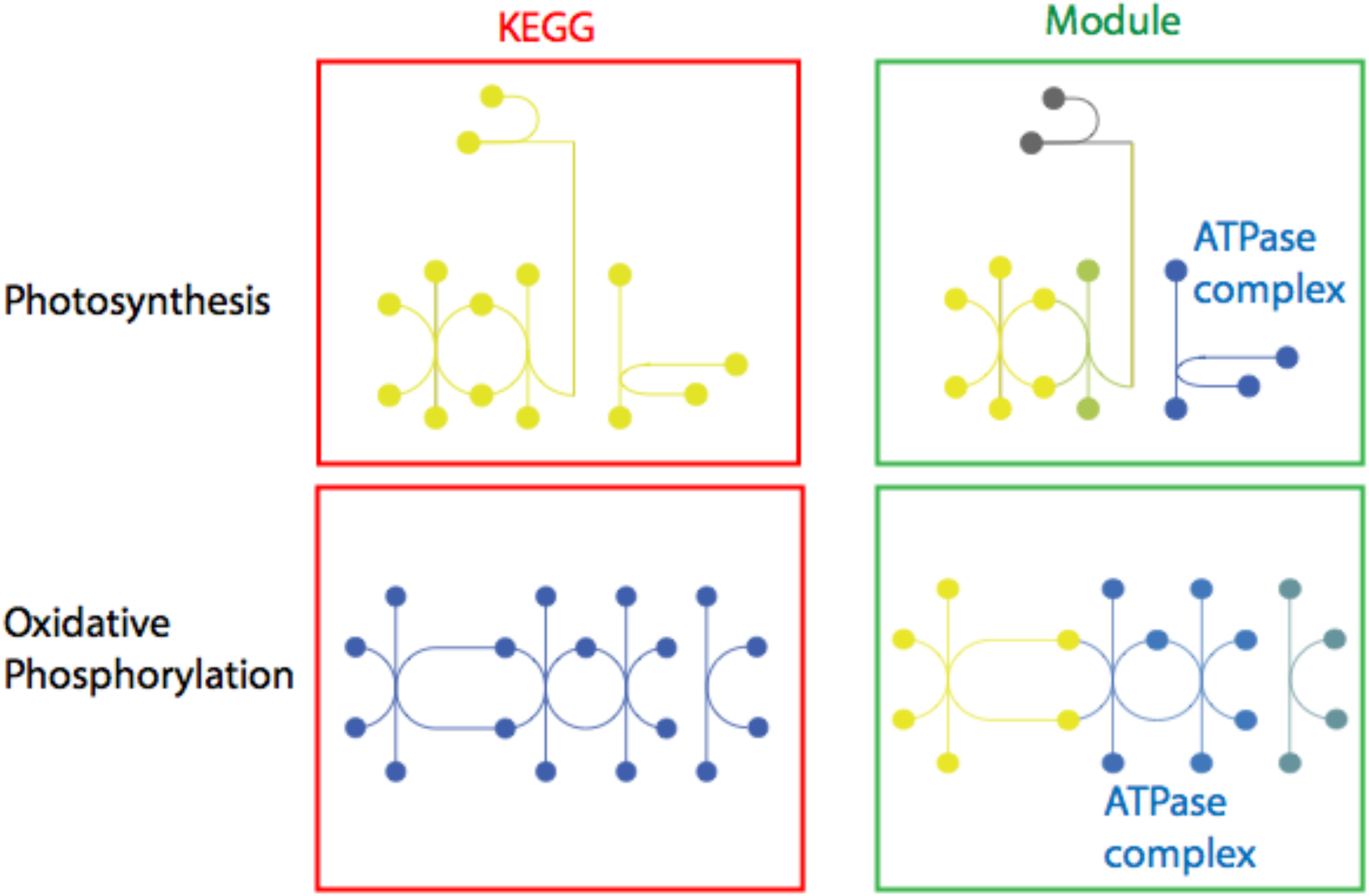
Environmentally variant



[Gianoulis et al., PNAS (in press, 2009)]

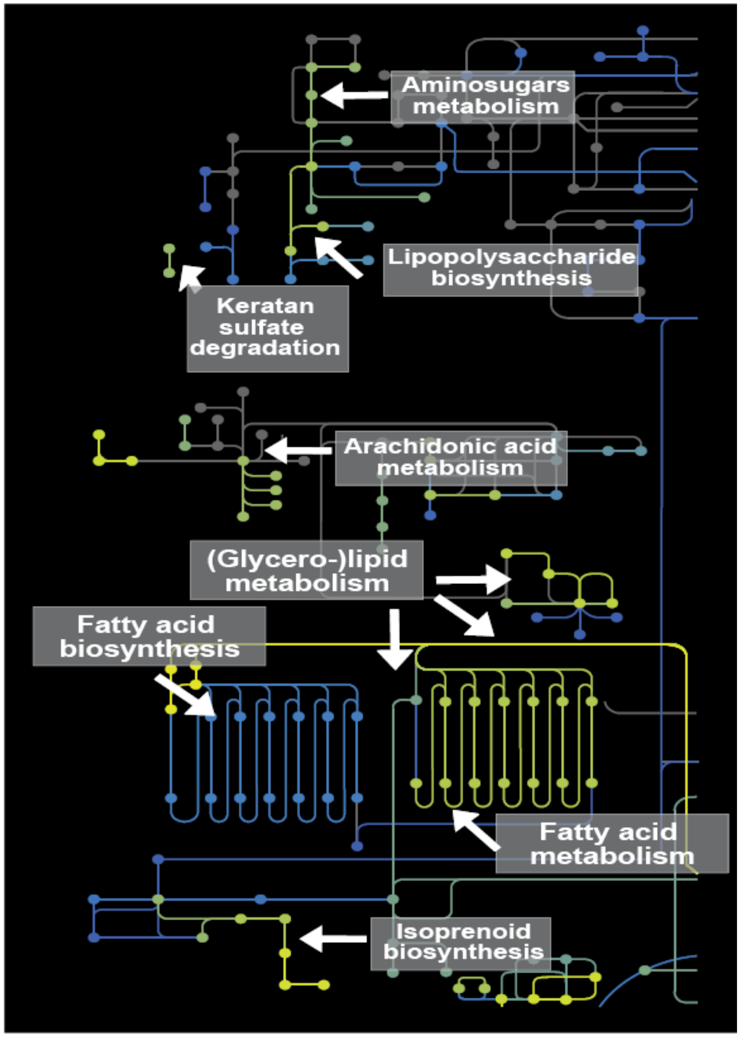
Conclusion #1: energy conversion strategy, temp and depth

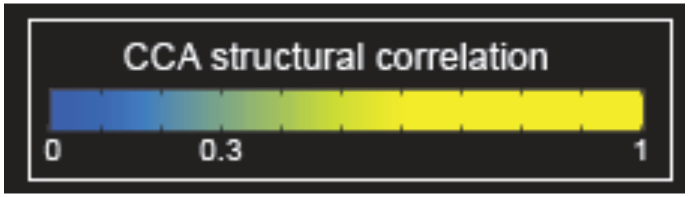




[Gianoulis et al., PNAS (in press, 2009)]

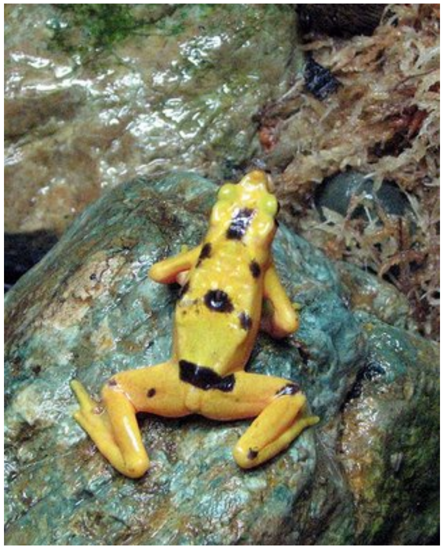
Conclusion #2: Outer Membrane components vary the environment


Aminosugars metabolism
Lipopolysaccharide biosynthesis
Keratan sulfate degradation
Arachidonic acid metabolism
(Glycero-)lipid metabolism
Fatty acid biosynthesis
Fatty acid metabolism
Isoprenoid biosynthesis



[Gianoulis et al., PNAS (in press, 2009)]

Biosensors:
Beyond Canaries in a Coal Mine



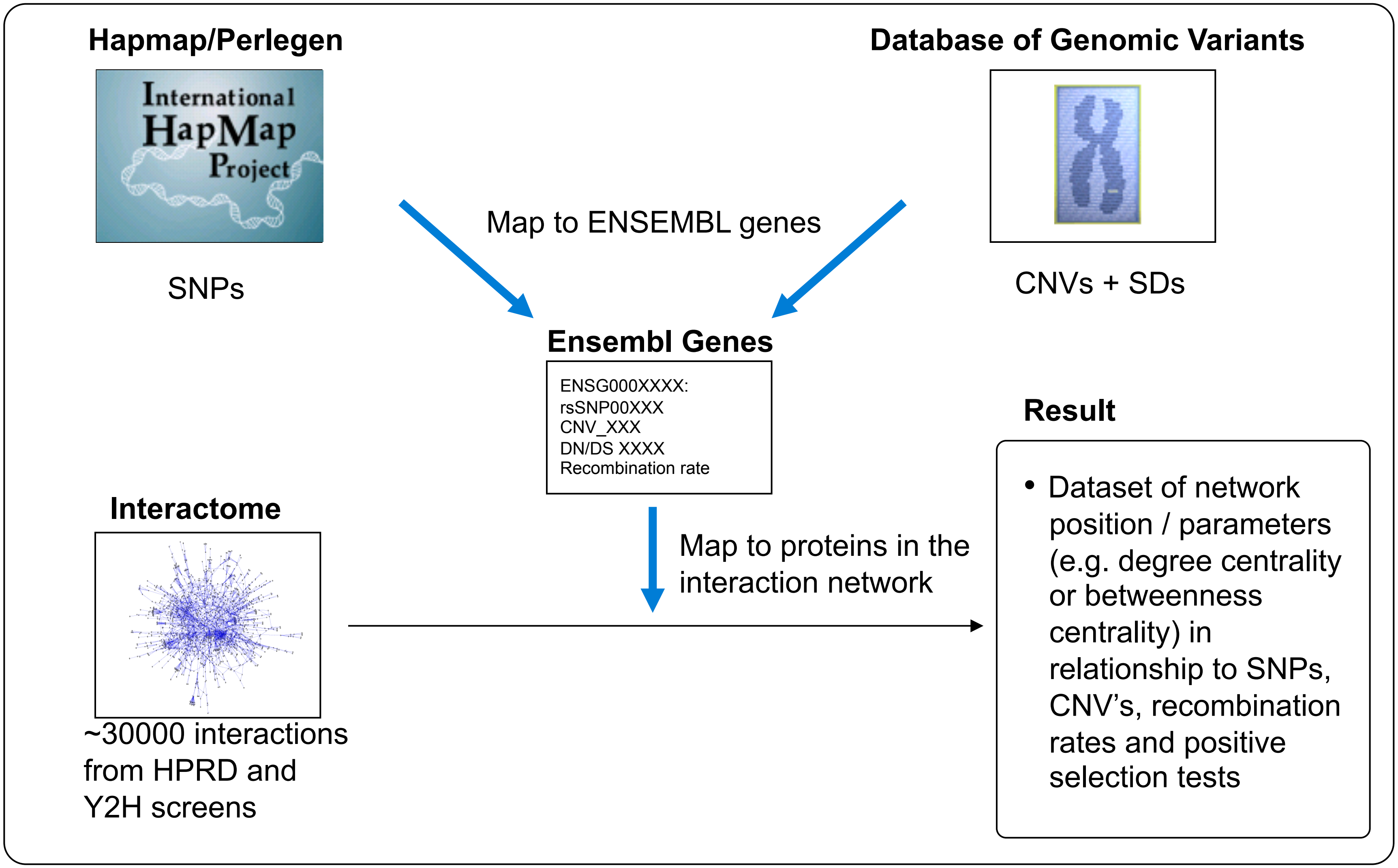
[Gianoulis et al., PNAS (in press, 2009)]

Networks & Variation

Which parts of the network vary most in sequence?
Which are under selection, either positive or negative?

METHODOLOGY: MAP SNP AND CNV DATA ONTO ENSEMBL GENES, AND THEN MAP ENSEMBL GENES TO THE KNOWN INTERACTOME

ILLUSTRATIVE



Hapmap/Perlegen

SNPs

Map to ENSEMBL genes

Database of Genomic Variants

CNVs + SDs

Ensembl Genes

ENSG000XXXX:
rsSNP00XXX
CNV_XXX
DN/DS XXXX
Recombination rate

Interactome

~30000 interactions from HPRD and Y2H screens

Map to proteins in the interaction network

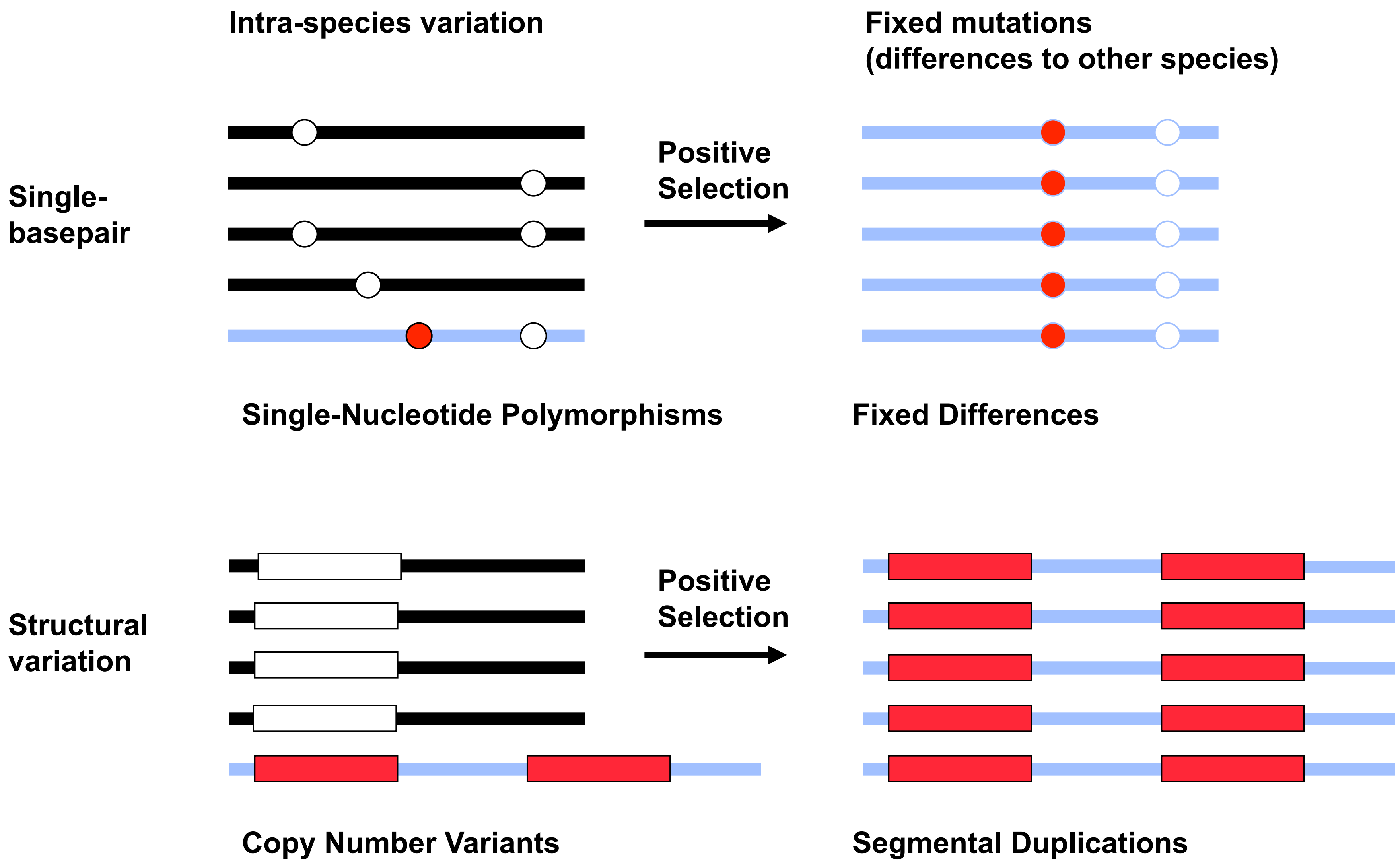
Result

- Dataset of network position / parameters (e.g. degree centrality or betweenness centrality) in relationship to SNPs, CNV’s, recombination rates and positive selection tests

* From Nielsen et al. *PLoS Biol.* (2005) and Bustamante et al. *Nature* (2005)

Source: PMK

ADAPTIVE EVOLUTION CAN BE SEEN ON TWO DIFFERENT LEVELS



Intra-species variation

Fixed mutations
(differences to other species)

Single-
basepair

Positive
Selection

Single-Nucleotide Polymorphisms

Fixed Differences

Structural
variation

Positive
Selection

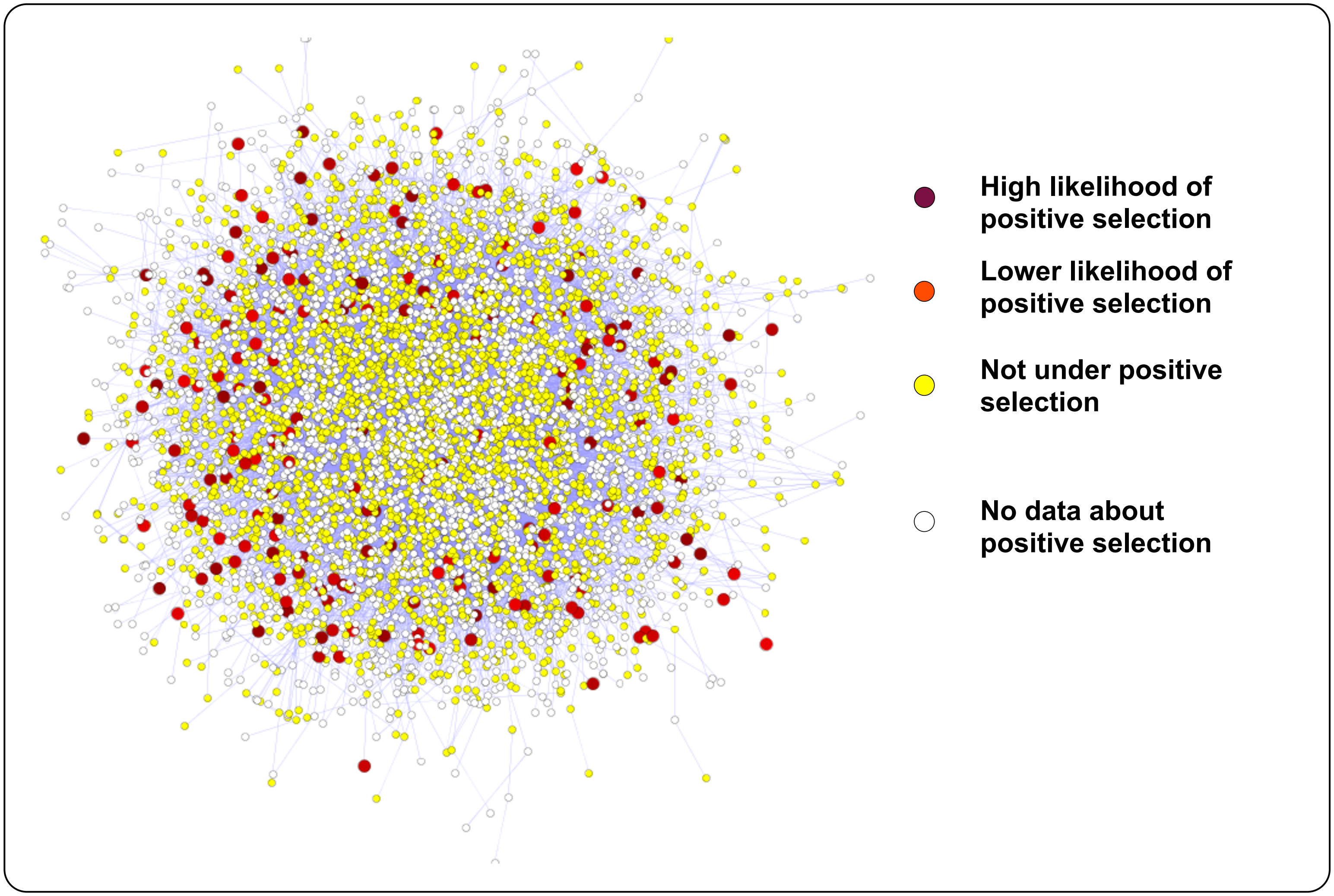
Copy Number Variants

Segmental Duplications

Source: PMK

POSITIVE SELECTION LARGELY TAKES PLACE AT THE NETWORK PERIPHERY

Positive selection in the human interactome

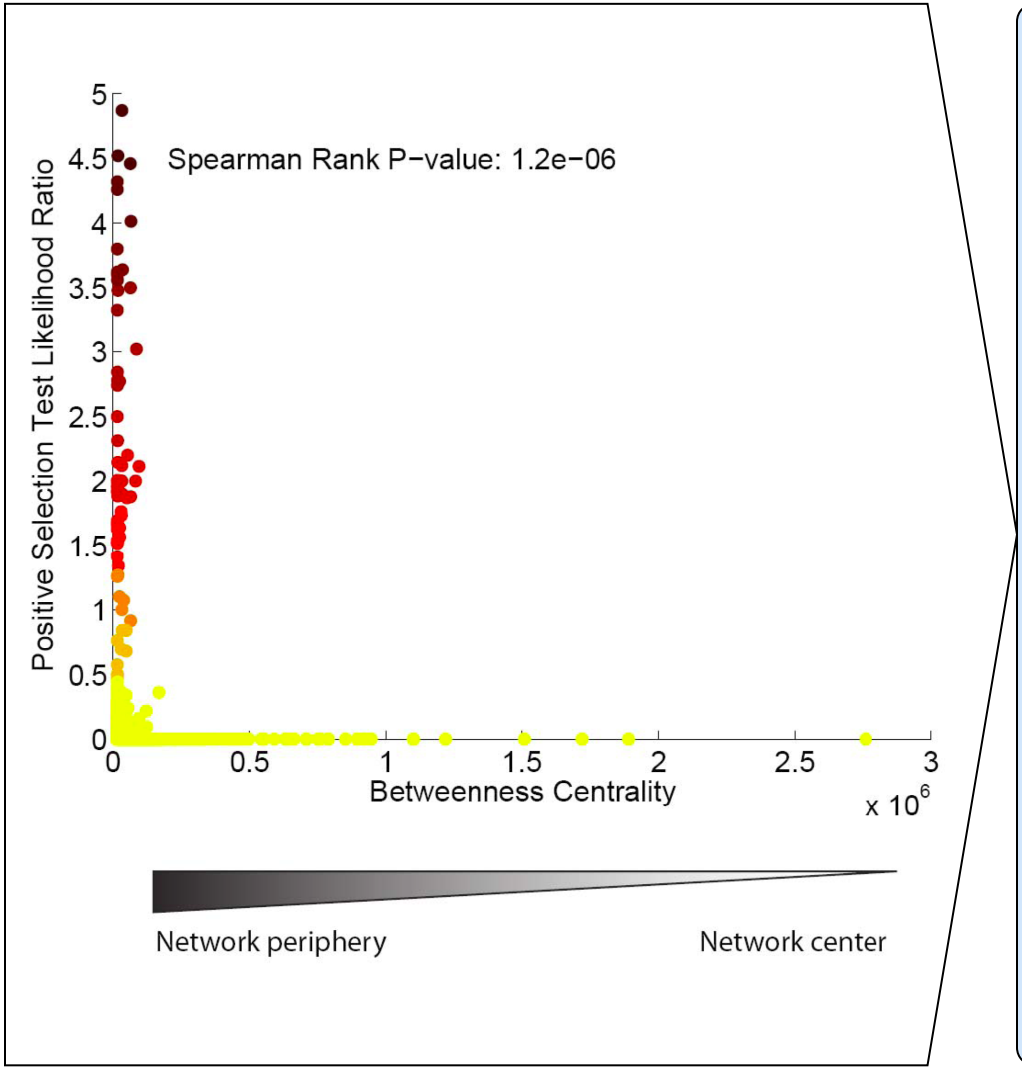

● High likelihood of positive selection
● Lower likelihood of positive selection
● Not under positive selection
○ No data about positive selection

Source: Nielsen et al. *PLoS Biol.* (2005), HPRD, and Kim et al. PNAS (2007)

CENTRAL PROTEINS ARE LESS LIKELY TO BE UNDER POSITIVE SELECTION

🟦 Hubs

Degree vs. Positive Selection



Reasoning

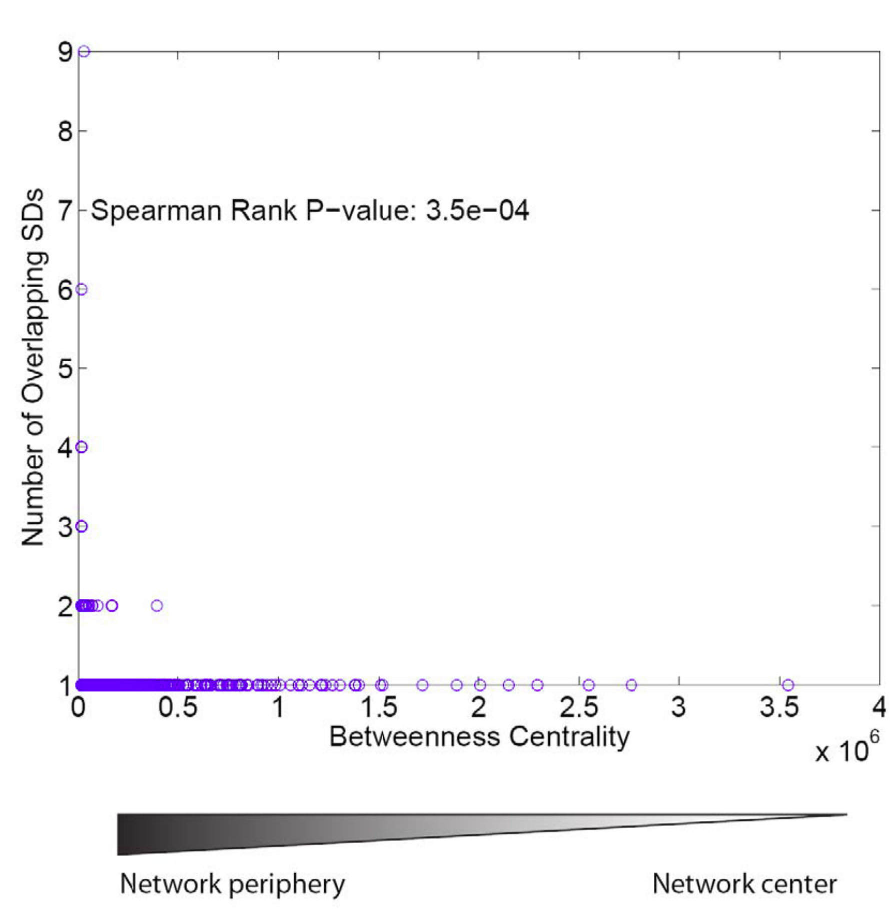
- Peripheral genes are likely to under positive selection, whereas hubs aren't
- This is likely due to the following reasons:
  - Hubs have stronger structural constraints, the network periphery doesn't
  - Most recently evolved functions (e.g. "environmental interaction genes" such as sensory perception genes etc.) would probably lie in the network periphery
- Effect is independent of any bias due to gene expression differences

* With a probability of over 80% to be positively selected as determined by Ka/Ks. Other tests of positive selection (McDonald Kreitmann and LDD) corroborate this result.

Source: Nielsen et al. *PLoS Biol.* (2005), Bustamante et al. *Nature* (2005), HPRD, Rual et al. *Nature* (2005), and Kim et al. PNAS (2007)

CENTRAL NODES ARE LESS LIKELY TO LIE INSIDE OF SDs

Centrality vs. SD occurrence



Reasoning

- This result also confirms our initial hypothesis – peripheral nodes tend to lie in regions rich in SDs.
- Since segmental duplications are a different mechanism of ongoing evolution, the less constrained peripheral proteins are enriched in them.
- Note that despite the small size of our dataset for known SD's we get significant correlations. It is to be expected that the correlations will get clearer as more data emerges*

* Specifically, a number of the SDs are likely not fixed, but rather common CNVs in the reference genome

Source: Database of genetic variation, HPRD, Rual et al. *Nature* (2005), and Kim et al. PNAS (2007)

IS RELAXED CONSTRAINT OR ADAPTIVE EVOLUTION THE REASON FOR THE PREVALENCE OF BOTH SELECTED GENES AND SDs AT THE NETWORK PERIPHERY?

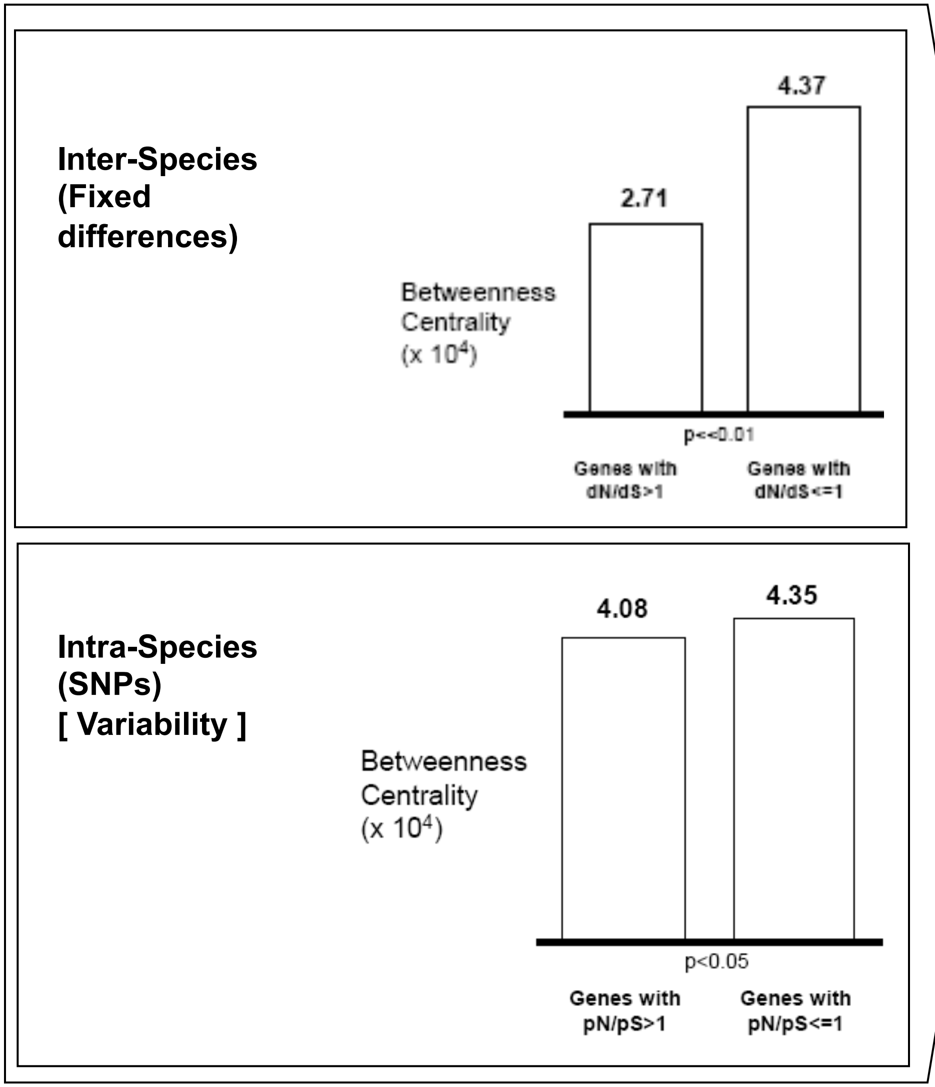
ILLUSTRATIVE

	Relaxed Constraint	**Adaptive Evolution**
Inter-Species Variation (Fixed differences)	• Increases inter-species variation – more variable loci are under less negative selection • Can be seen in higher Ka/ Ks ratio or SD occurrence	• Increases inter-species variation – more variable loci are under less negative selection • Can be seen in higher Ka/ Ks ratio or SD occurrence
Intra-Species Variation (Polymorphisms)	• Increases intra-species variation – for the very same reason • Can be seen in both SNPs or CNVs	• Should not have effects on intra-species variation

Source: Kim et al. PNAS (2007)

SOME, BUT NOT ALL OF THE SINGLE-BASEPAIR SELECTION AT THE PERIPHERY IS DUE TO RELAXED CONSTRAINT

Inter vs. Intra-Species Variation in Networks



Reasoning

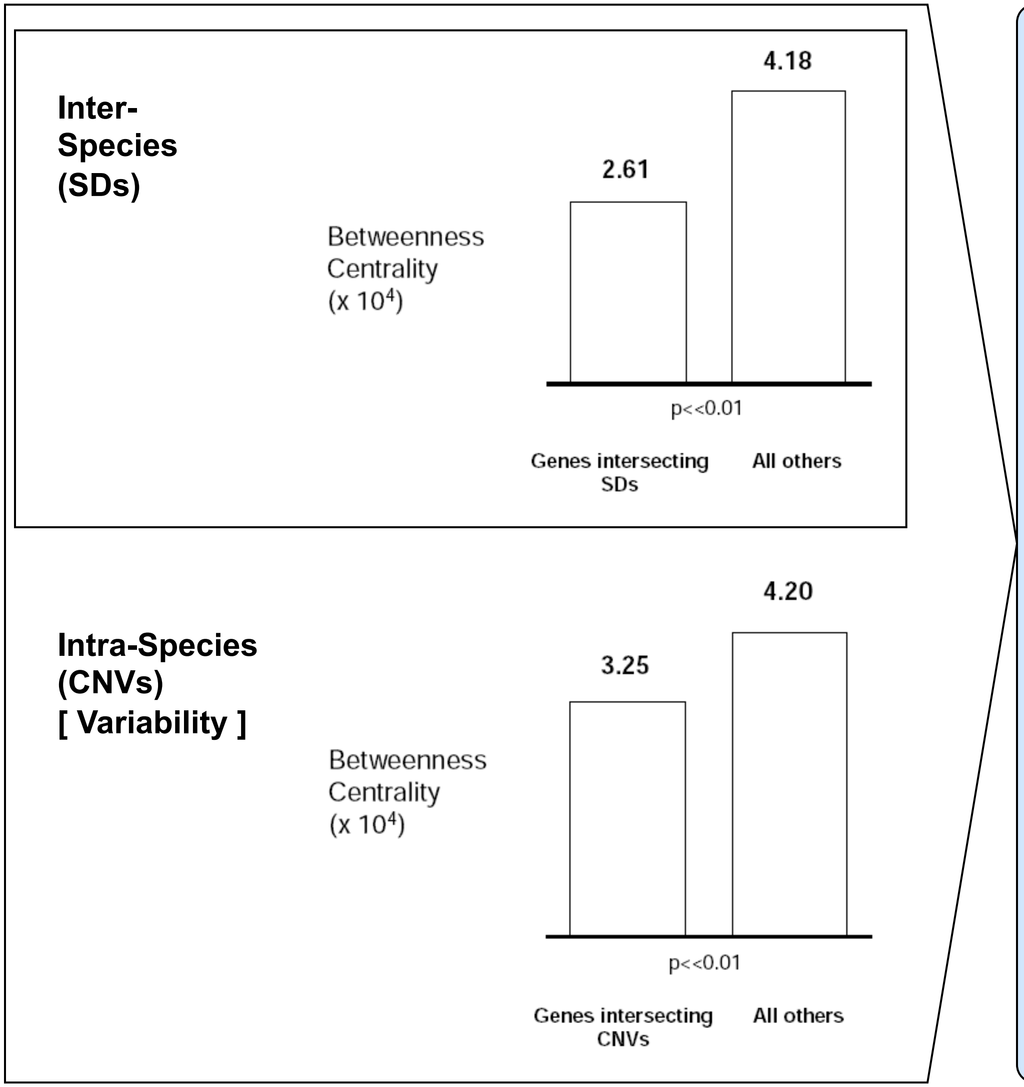
- There is a difference in **variability** (in terms of SNPs) between the network periphery and the center
- However, this difference is much smaller than the difference in **selection**
- This most likely means, that part of the effect we're seeing is due to relaxed constraint (and higher variability)
- But, not the entire effect*

* But it's hard to quantify

Source: Kim et al. (2007) PNAS

Similar Results for Large-scale Genomic Changes (CNVs and SDs)

Inter vs. Intra-Species Variation in Networks



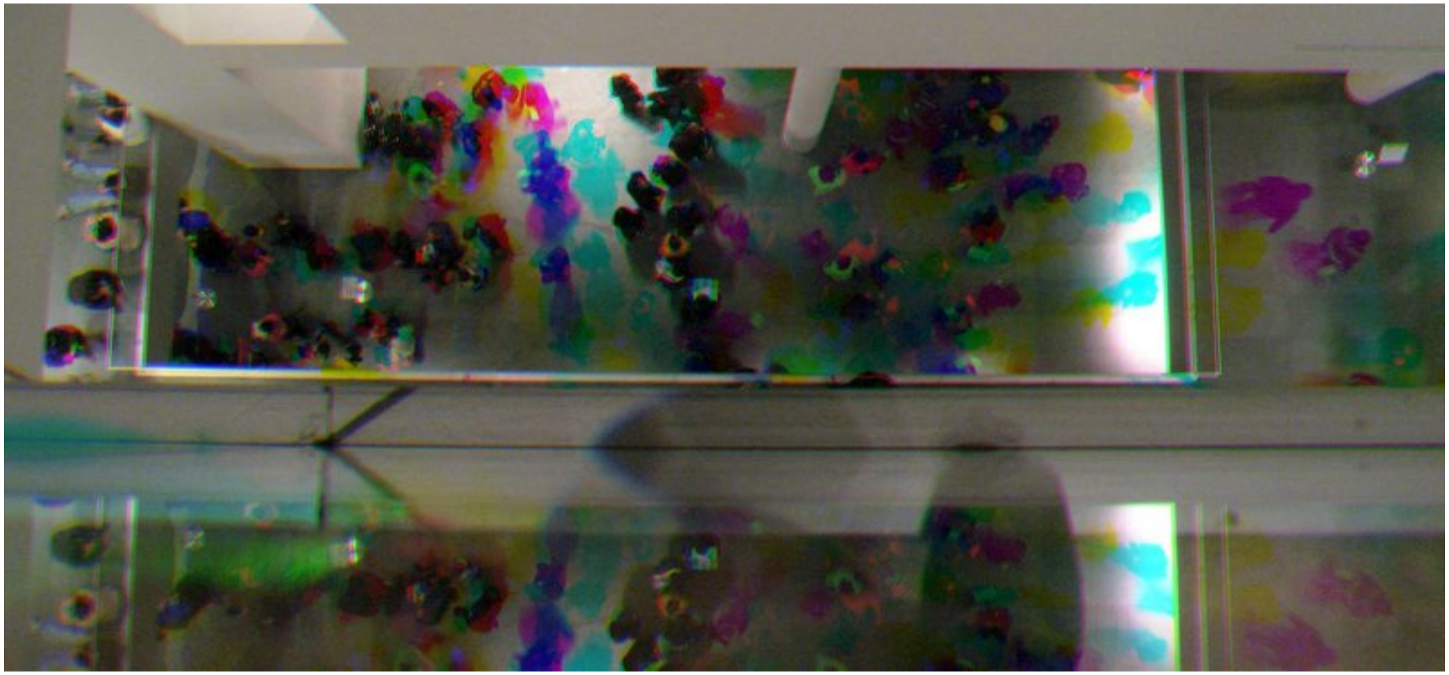
Reasoning

- There a small difference in **variability** (in terms of CNVs) between the network periphery and the center
- But, there is a (as shown before) marked difference in fixed (and hence, presumably, **selected**) SDs at the network periphery and center

Source: Kim et al. (2007) PNAS

Networks & Variation 2

Variation in the miRNA network

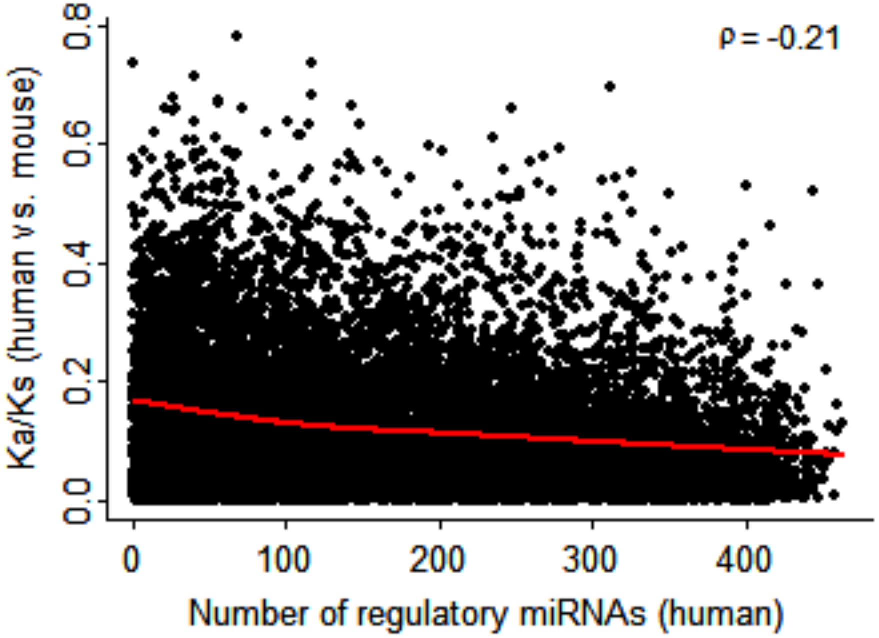


Analyze Regulation in microRNA-target Network

- Relationship between target in degree (number of micro-RNAs that regulate gene) & evolutionary rate of gene?
  - ◊ In deg. related 3' UTR size

- Expectation: more regulation, more constraint

Relationship between microRNA regulation and protein evolution



Human vs.	Number of genes	Correlation	P-value
chimpanzee	11326	-0.11	2.E-32
mouse	13280	-0.21	7.E-128
rat	12270	-0.20	4.E-107
cow	11683	-0.21	8.E-115
chicken	8061	-0.18	1.E-57

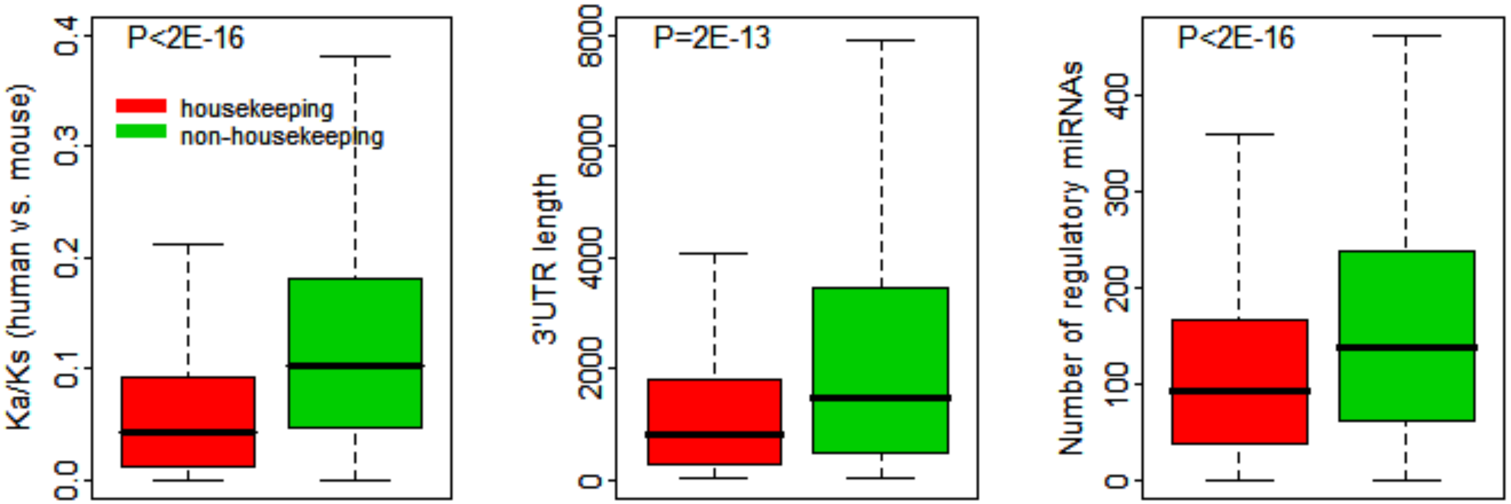
Important genes are regulated more intensively regulated by the microRNAs

[Cheng et al., BMC Genomics, 2009 (in press)]

MicroRNA regulation: a two-way strategy

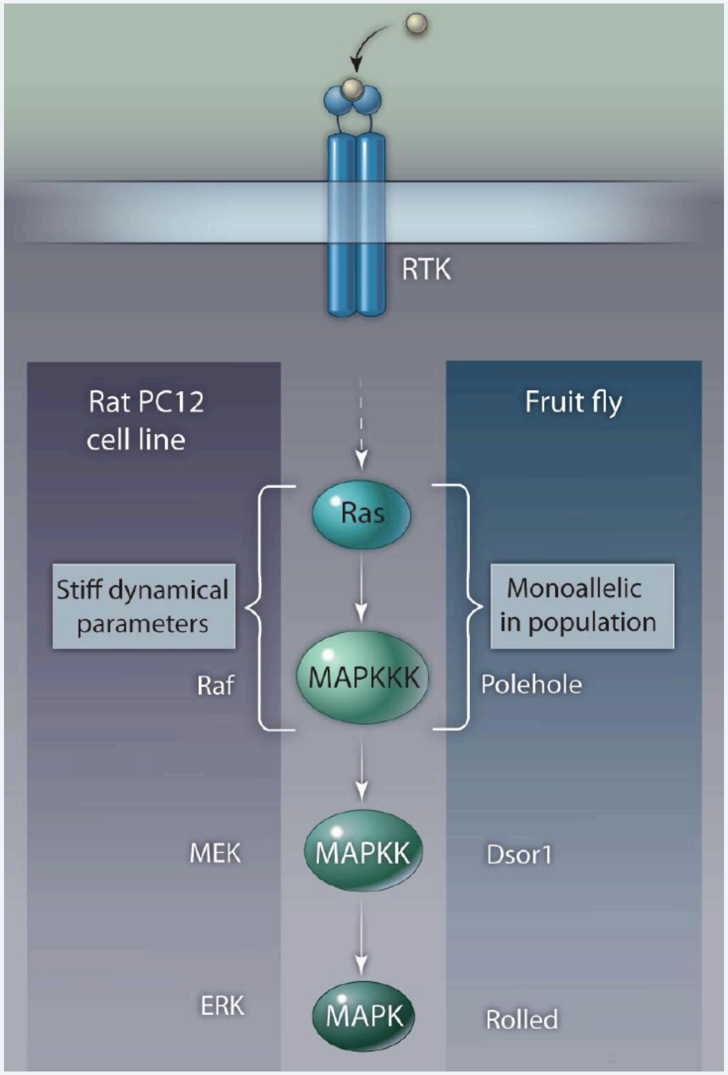
For non-housekeeping genes, functionally critical genes are intensively regulated by miRNAs and prefer long 3'UTR.

housekeeping genes, however conserved, are selected to have shorter 3'UTRs to avoid miRNA regulation.



[Cheng et al., BMC Genomics, 2009 (in press)]

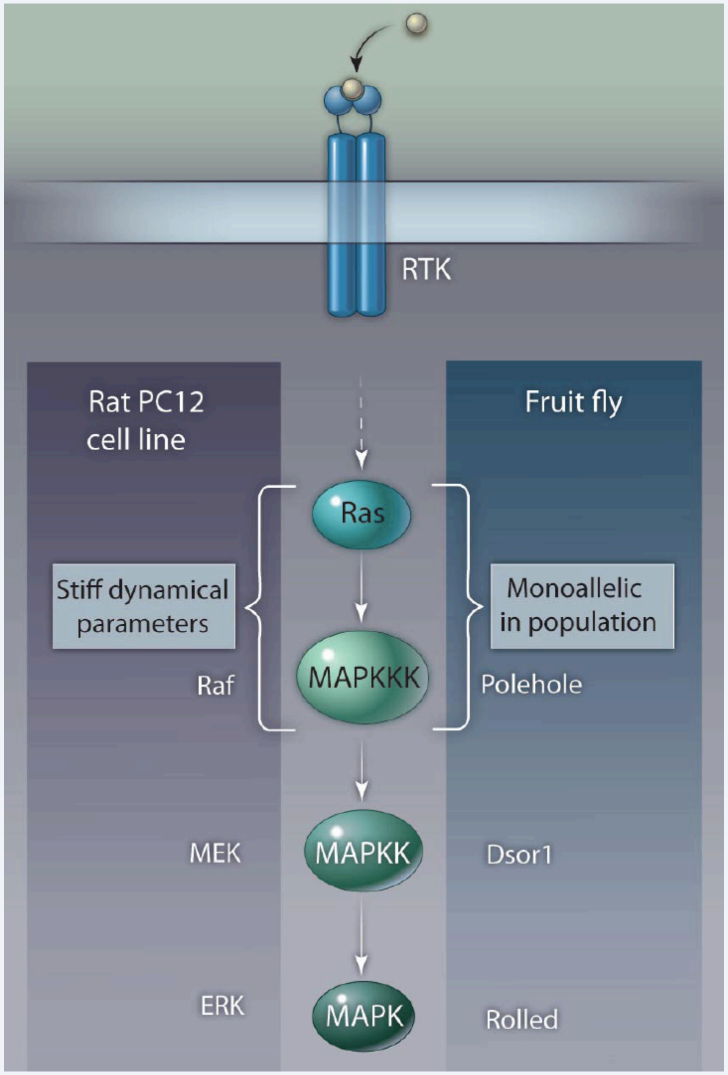
Network dynamics constrain evolution



Hypothesis: Nodes in a molecular network with the strongest impact on dynamic behavior should be under strong purifying selection and thus exhibit the least genetic variation.

Alexander et al. *Sci. Signal.* (2009) 2: pe44

Network dynamics constrain evolution

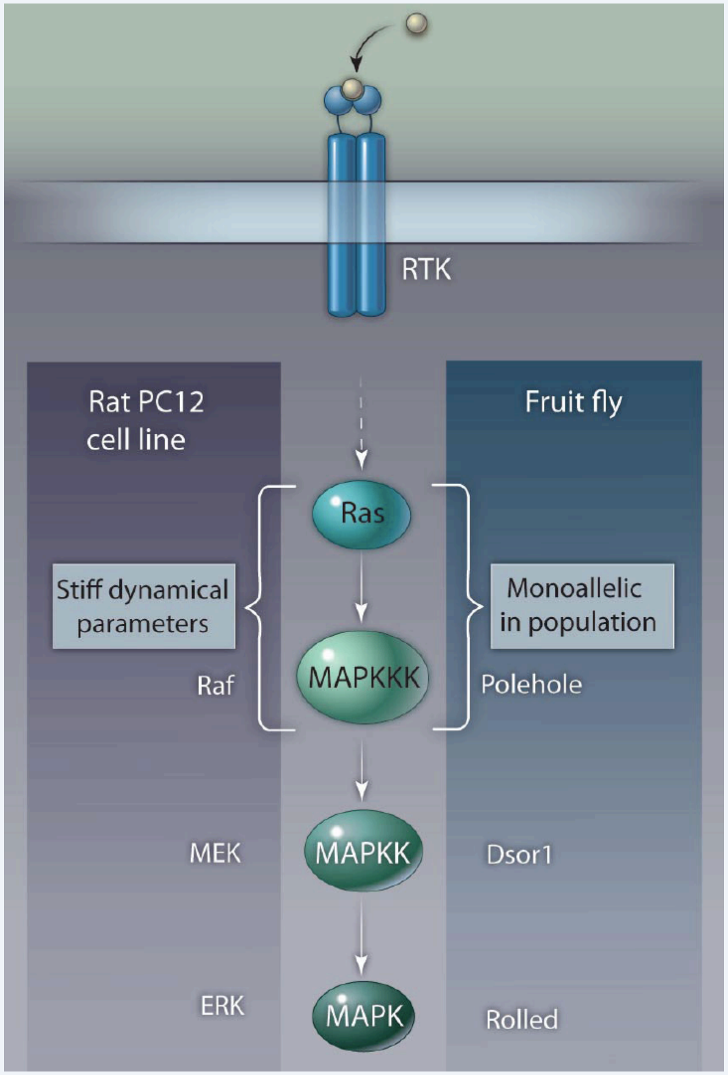


Hypothesis: Nodes in a molecular network with the strongest impact on dynamic behavior should be under strong purifying selection and thus exhibit the least genetic variation.

Algorithm:
1) Reconstruct families of molecular networks from genomic data.
2) Map some kind of genetic variation onto the networks.
3) Analyze sensitivity of dynamical model of the generic network.

Alexander et al. *Sci. Signal.* (2009) 2: pe44

Speculation: Why more tightly regulated gene might have less variation



Example: MAP Kinase singaling pathway

Dynamic model:

- ODE model with Michaelis-Menten kinetics
- parameters fit to time series data of protein activities in response to EGF and NGF from rat PC12 cell line

In sensitivity analysis, stiff parameters cluster around Ras and Raf.

Population study in fruit flies:

- allele variation based on PCR of pathway genes

Ras and Raf have less allele variation than other proteins in the network.

Brown et al. *Phys. Biol.* (2004) 1: 184
Riley et al. *Molec. Ecol.* (2003) 12: 1315

Alexander et al*. Sci. Signal.* (2009) 2: pe44

- Why Networks?
- Generating Networks
  - ◊ Processing Protein Chips
    (yeast & human nets)
  - ◊ Propagating Known Information
    (yeast ppi)
- Central Points in Networks
  - ◊ Hubs & Bottlenecks
    (yeast ppi & reg. net)
  - ◊ Tops of Heirarchies
    (yeast reg. net)
  - ◊ Identified by score
    (human miRNA-targ. net)
- Dynamics of Networks
  - ◊ Across environments
    (prokaryote metab. pathways)
- Protein Networks & Variation
  (human ppi & miRNA-targ. net)

Outline: Molecular Networks

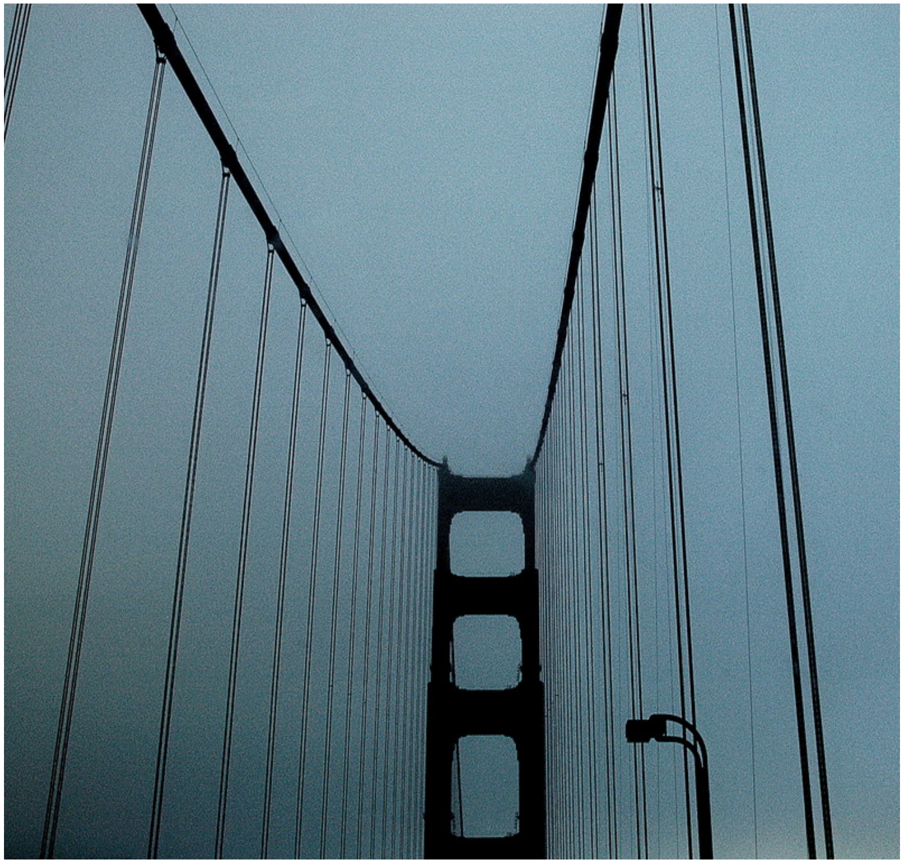
Conclusions on Networks: Generation

- Networks from processing protein chip data
  - ◊ RLM normalization surpresses quantile
- Predicting Networks
  - ◊ Extrapolating from the Training Set
  - ◊ Principled ways of using known information in the fullest possible fashion
    - Multi-level learning

Conclusions: Analysis of Network Structure

- Centrality Measures in Protein Network
  - ◊ Hubs & Bottlenecks
  - ◊ Importance of later in regulatory networks
- Regulatory Network Hierarchies
  - ◊ Middle managers dominate, sitting at info. flow bottlenecks
  - ◊ Paradox of influence & essentiality

Conclusions:
Points of Network Centrality



- RE-score measures degree of (down) regulation of targets v. non-targets
- Application to miRNA network
- Use in cancer classification

Conclusions: Networks Dynamics across Environments

- Developed and adapted techniques to connect quantitative features of environment to metabolism.
- Applied to available aquatic datasets, we identified footprints that were predictive of their environment (potentially could be used as biosensor).
- Strong correlation exists between a community's energy conversion strategies and its environmental parameters (e.g. temperature and chlorophyll).
- Suggest that limiting amounts of cofactor can (partially) explain increased import of amino acids in nutrient-limited conditions.

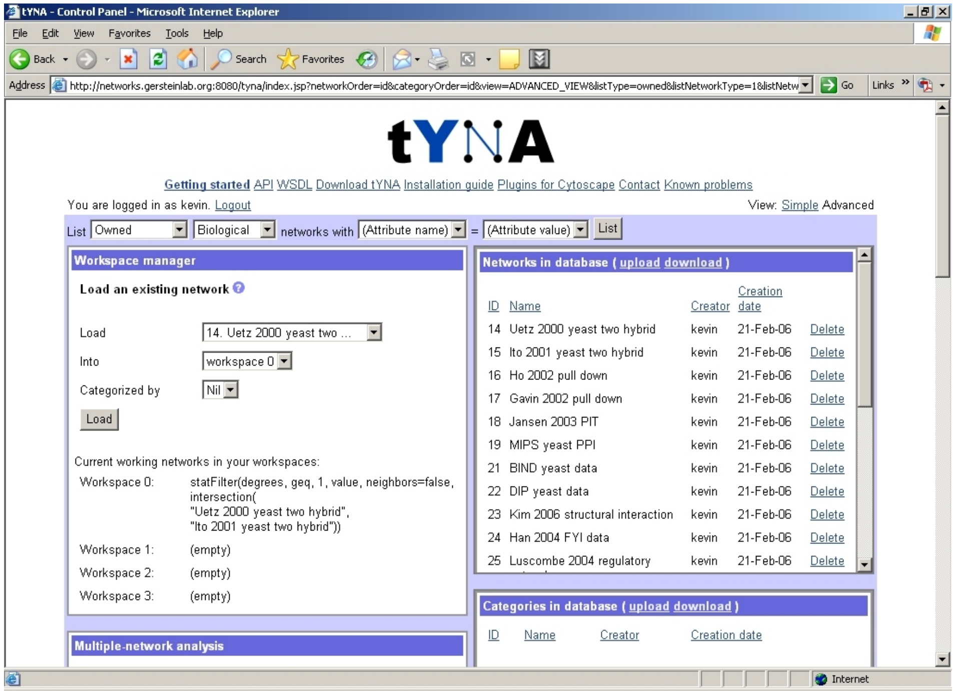
Conclusions: Connecting Networks & Variation

- Positive selection (adaptive evolution) at the network periphery
  - ◊ On a sequence level, it can be seen as positive selection of peripheral nodes
  - ◊ On a structural level, it can be seen as the pattern of SDs that display significantly higher allele frequencies in non-central genes
- miRNA network
  - ◊ More highly regulated genes are under more constraint in miRNA-target networks
  - ◊ Exception for housekeeping genes

- an automated web tool

tYNA

(vers. 2 : "TopNet-like Yale Network Analyzer")



tYNA - Control Panel - Microsoft Internet Explorer

http://networks.gersteinlab.org:8080/tyna/index.jsp?networkOrder=id&categoryOrder=id&view=ADVANCED_VIEW&listType=owned&listNetworkType=1&listNetw

tYNA

Getting started API WSDL Download tYNA Installation guide Plugins for Cytoscape Contact Known problems

You are logged in as kevin. Logout

View: Simple Advanced

List Owned Biological networks with (Attribute name) = (Attribute value) List

Workspace manager

Load an existing network

Load: 14. Uetz 2000 yeast two ...

Into: workspace 0

Categorized by: Nil

Load

Current working networks in your workspaces:

Workspace 0:	statFilter(degrees, geq, 1, value, neighbors=false, intersection("Uetz 2000 yeast two hybrid", "Ito 2001 yeast two hybrid"))
Workspace 1:	(empty)
Workspace 2:	(empty)
Workspace 3:	(empty)

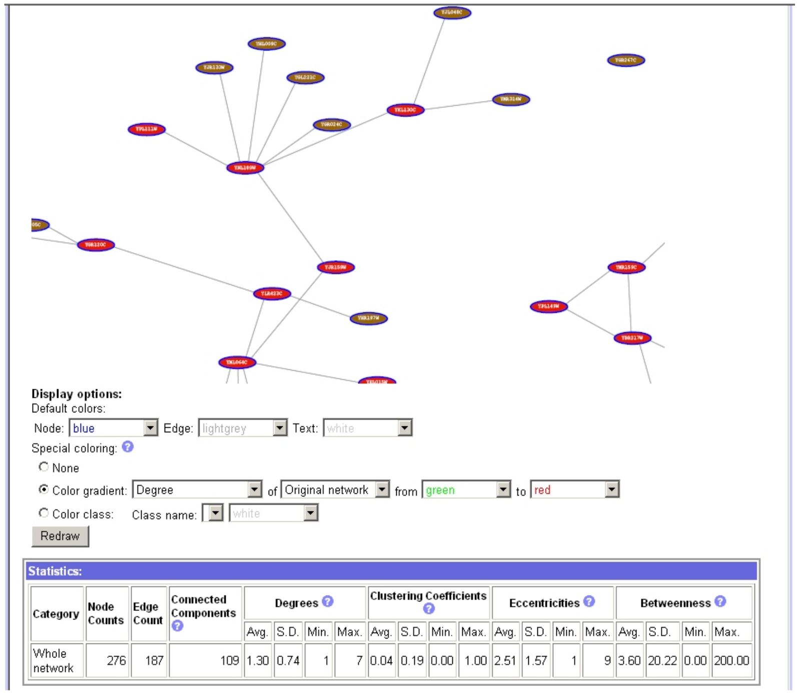
Multiple-network analysis

Networks in database (upload download)

ID	Name	Creator	Creation date	
14	Uetz 2000 yeast two hybrid	kevin	21-Feb-06	Delete
15	Ito 2001 yeast two hybrid	kevin	21-Feb-06	Delete
16	Ho 2002 pull down	kevin	21-Feb-06	Delete
17	Gavin 2002 pull down	kevin	21-Feb-06	Delete
18	Jansen 2003 PIT	kevin	21-Feb-06	Delete
19	MIPS yeast PPI	kevin	21-Feb-06	Delete
21	BIND yeast data	kevin	21-Feb-06	Delete
22	DIP yeast data	kevin	21-Feb-06	Delete
23	Kim 2006 structural interaction	kevin	21-Feb-06	Delete
24	Han 2004 FYI data	kevin	21-Feb-06	Delete
25	Luscombe 2004 regulatory	kevin	21-Feb-06	Delete

Categories in database (upload download)

ID	Name	Creator	Creation date



Display options:

Default colors:

Node: blue Edge: lightgrey Text: white

Special coloring:

○ None

● Color gradient: Degree of Original network from green to red

○ Color class: Class name: white

Redraw

Statistics:

Category	Node Counts	Edge Count	Connected Components	Degrees				Clustering Coefficients				Eccentricities				Betweenness			
				Avg.	S.D.	Min.	Max.	Avg.	S.D.	Min.	Max.	Avg.	S.D.	Min.	Max.	Avg.	S.D.	Min.	Max.
Whole network	276	187	109	1.30	0.74	1	7	0.04	0.19	0.00	1.00	2.51	1.57	1	9	3.60	20.22	0.00	200.00

Normal website + Downloaded code (JAVA)
+ Web service (SOAP) with Cytoscape plugin

[Yu et al., NAR (2004); Yip et al. Bioinfo. (2006);
Similar tools include Cytoscape.org, Idekar, Sander et al]

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P Alves
N Bhardwaj
R Alexander
P Cayting
M Seringhaus
Y Xia
J Korbel
E Franzosa

J Raes
T Emonet
P Bork
B Schweitzer
M Snyder

Networks.GersteinLab.org

Job opportunities currently for postdocs & students

More Information on this Talk

SUBJECT: Networks

DESCRIPTION:

CSHL, Cold Spring Harbor, NY; 2010.01.06, 12:00-13:00; [I:**CSHL2**] (Long networks talk, derived from [I:**MBINETS**], including **rlm*** & new intro. for 1st time)

(PPT works on mac & PC and has many photos. Paper references in the talk were mostly from Papers.GersteinLab.org. The above topic list can be easily cross-referenced against this website. Each topic abbrev. which is starred is actually a papers "ID" on the site. For instance, the topic **pubnet*** can be looked up at **http://papers.gersteinlab.org/papers/pubnet**)

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