Bioinformatics 3

V6 – Biological PPI Networks
- are they really scale-free?
- network growth
- functional annotation in the network

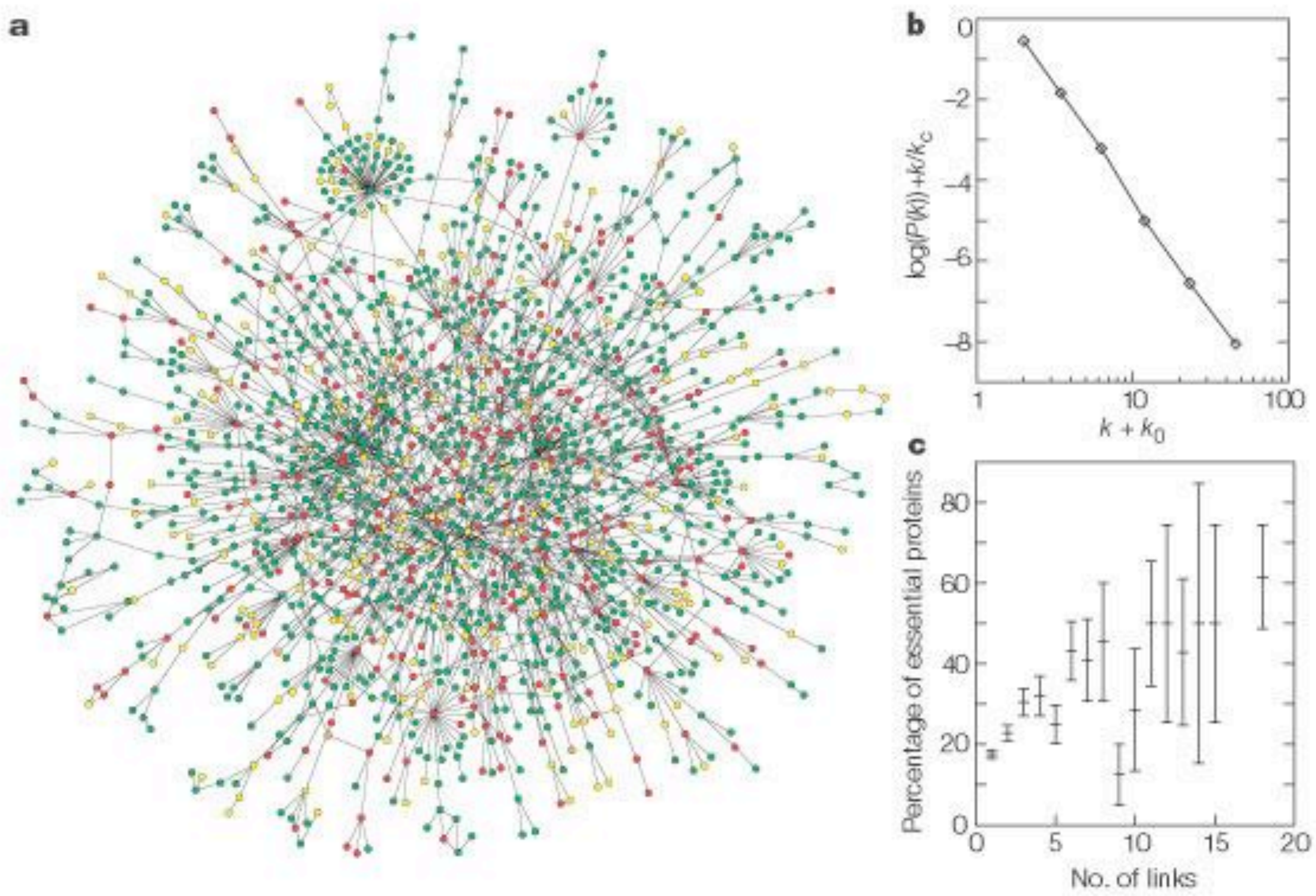
Mon, Nov 10, 2014

brief communications

Lethality and centrality in protein networks

The most highly connected proteins in the cell are the most important for its survival.

Jeong, Mason, Barabási, Oltvai, *Nature* **411** (2001) 41



largest cluster of the yeast proteome (at 2001)

→ "PPI networks apparently are scale-free…"

"Are" they scale-free

or

"Do they look like" scale-free???

Partial Sampling

Estimated for yeast: 6000 proteins, 30000 interactions

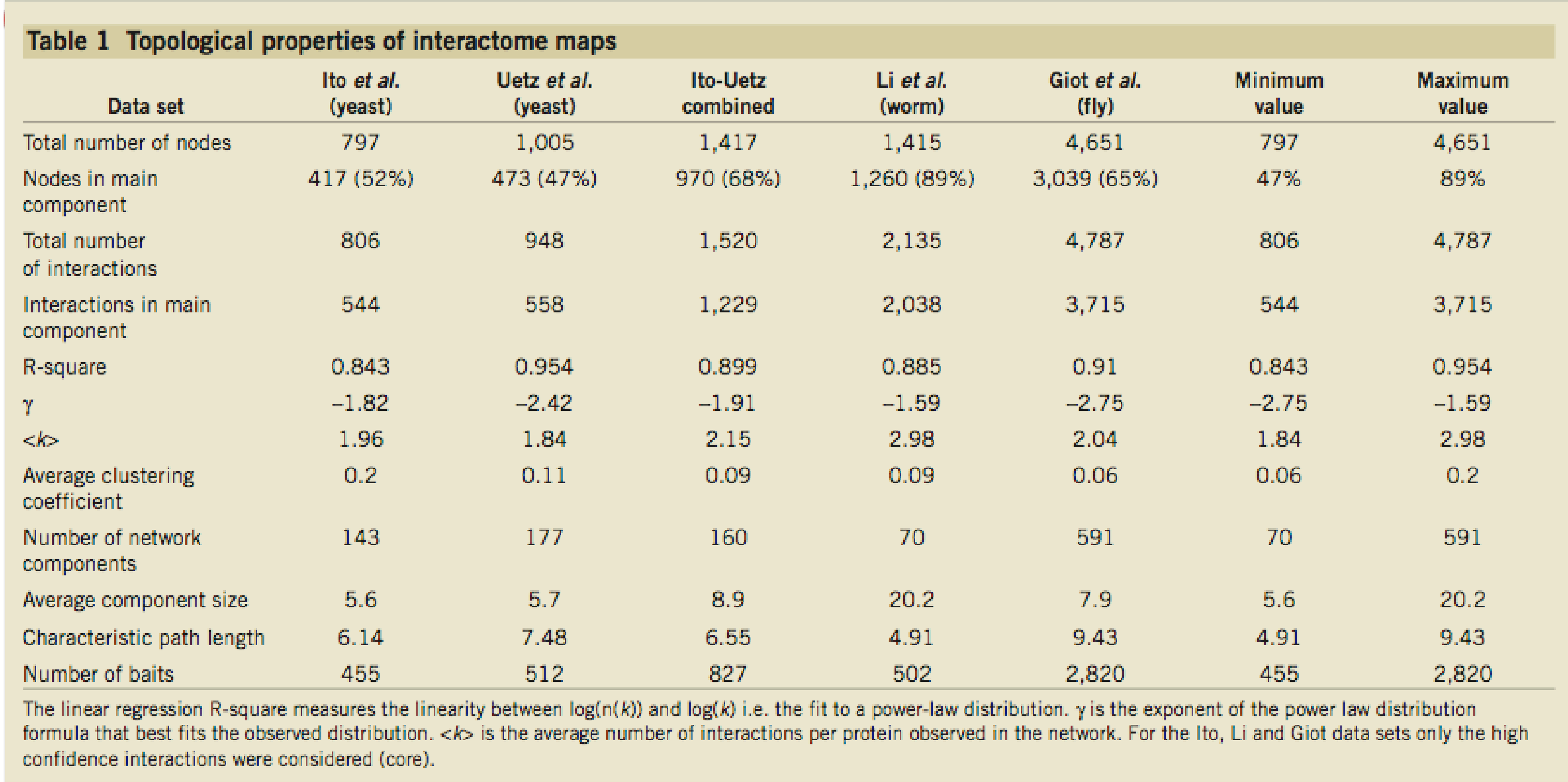


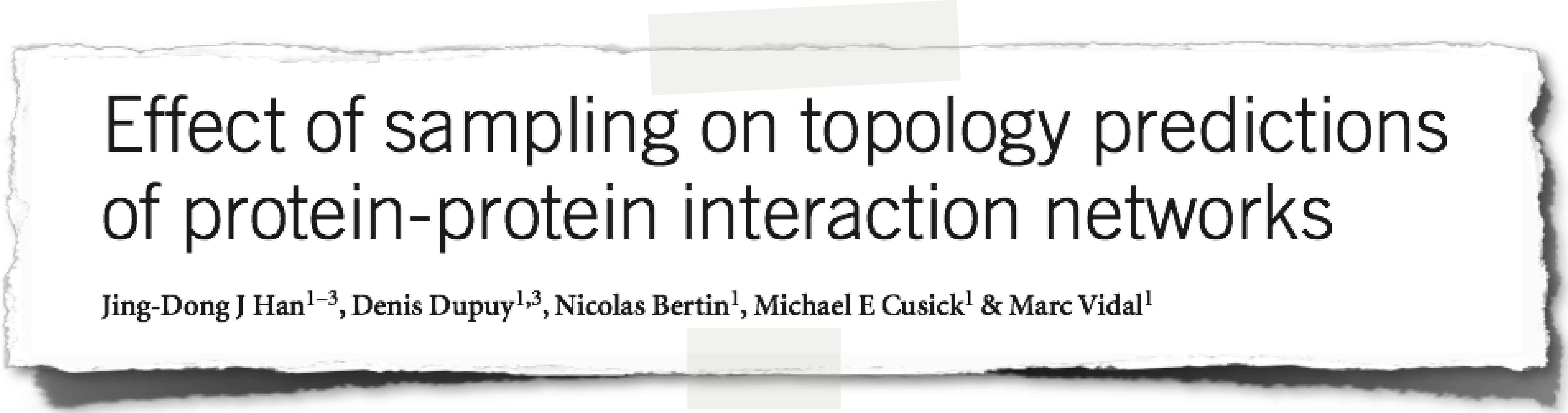
Table 1 Topological properties of interactome maps

Data set	Ito *et al.* (yeast)	Uetz *et al.* (yeast)	Ito-Uetz combined	Li *et al.* (worm)	Giot *et al.* (fly)	Minimum value	Maximum value
Total number of nodes	797	1,005	1,417	1,415	4,651	797	4,651
Nodes in main component	417 (52%)	473 (47%)	970 (68%)	1,260 (89%)	3,039 (65%)	47%	89%
Total number of interactions	806	948	1,520	2,135	4,787	806	4,787
Interactions in main component	544	558	1,229	2,038	3,715	544	3,715
R-square	0.843	0.954	0.899	0.885	0.91	0.843	0.954
γ	–1.82	–2.42	–1.91	–1.59	–2.75	–2.75	–1.59
<*k*>	1.96	1.84	2.15	2.98	2.04	1.84	2.98
Average clustering coefficient	0.2	0.11	0.09	0.09	0.06	0.06	0.2
Number of network components	143	177	160	70	591	70	591
Average component size	5.6	5.7	8.9	20.2	7.9	5.6	20.2
Characteristic path length	6.14	7.48	6.55	4.91	9.43	4.91	9.43
Number of baits	455	512	827	502	2,820	455	2,820

The linear regression R-square measures the linearity between log(n(*k*)) and log(*k*) i.e. the fit to a power-law distribution. γ is the exponent of the power law distribution formula that best fits the observed distribution. <*k*> is the average number of interactions per protein observed in the network. For the Ito, Li and Giot data sets only the high confidence interactions were considered (core).

Y2H **covers** only **3…9%** of the complete interactome!

Han et al, *Nature Biotech* **23** (2005) 839

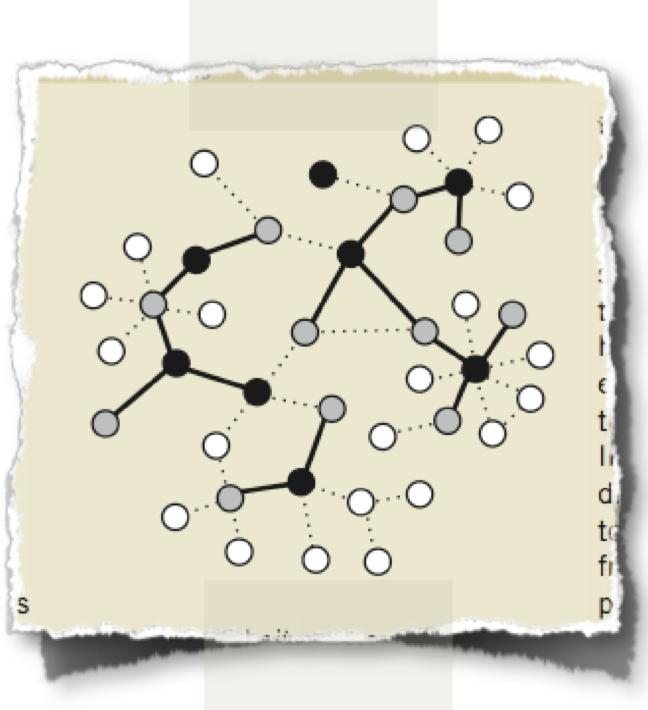


Effect of sampling on topology predictions of protein-protein interaction networks

Jing-Dong J Han[1–3], Denis Dupuy[1,3], Nicolas Bertin[1], Michael E Cusick[1] & Marc Vidal[1]

Nature Biotech **23** (2005) 839

Generate networks of various types,
sample sparsely from them
→ degree distribution?

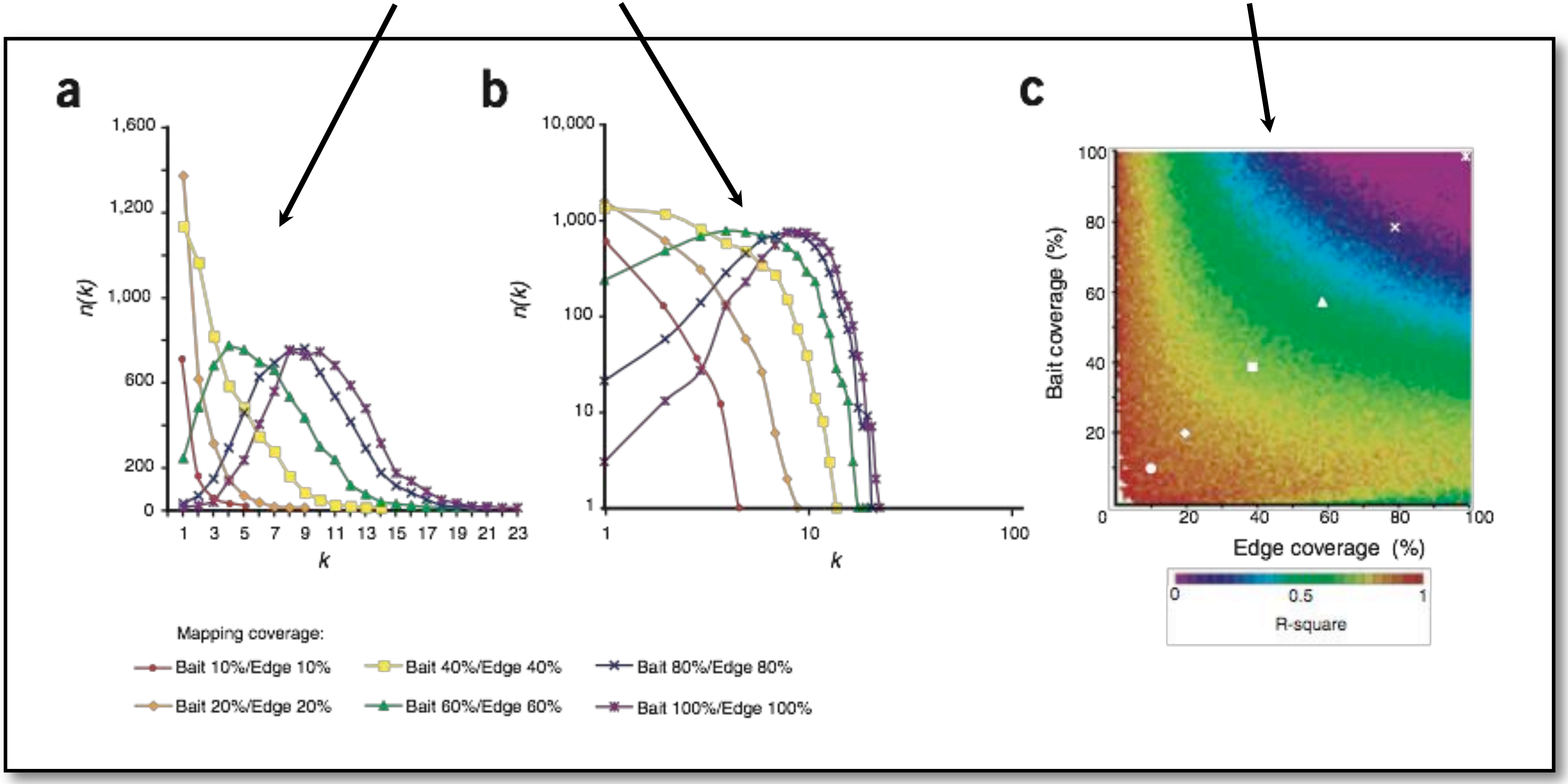


- Random (ER / Erdös-Renyi) → *P(k)* = Poisson
- Exponential (EX) → *P(k)* ~ exp[-*k*]
- scale-free / power-law (PL) → *P(k)* ~ $k^{-\gamma}$
- *P(k)* = truncated normal distribution (TN)

Sparsely Sampled random (ER) Network

resulting *P(k)* for different coverages

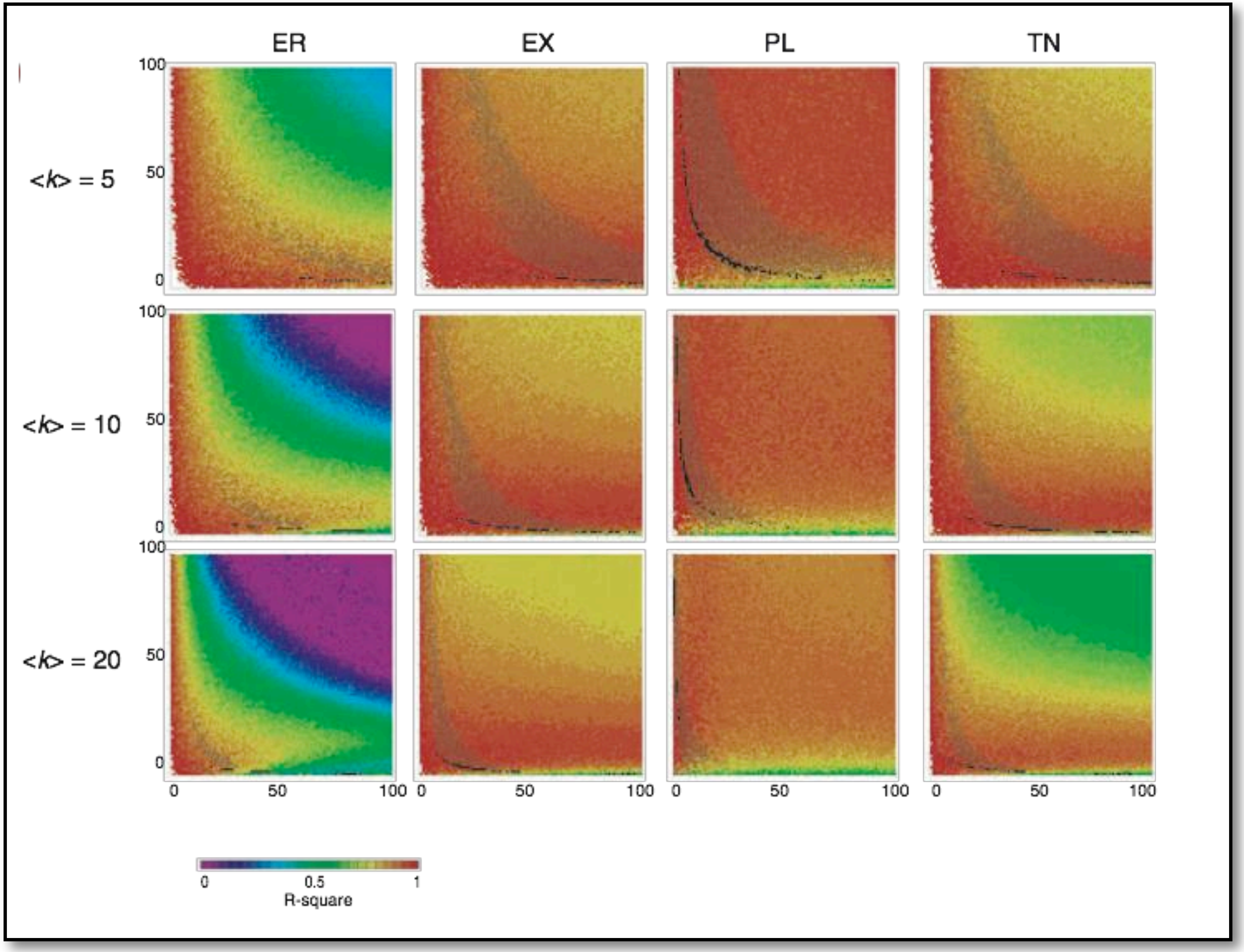
linearity between *P(k)* and power law



→ for **sparse** sampling, even an ER networks "**looks**" **scale-free** (when only *P(k)* is considered)

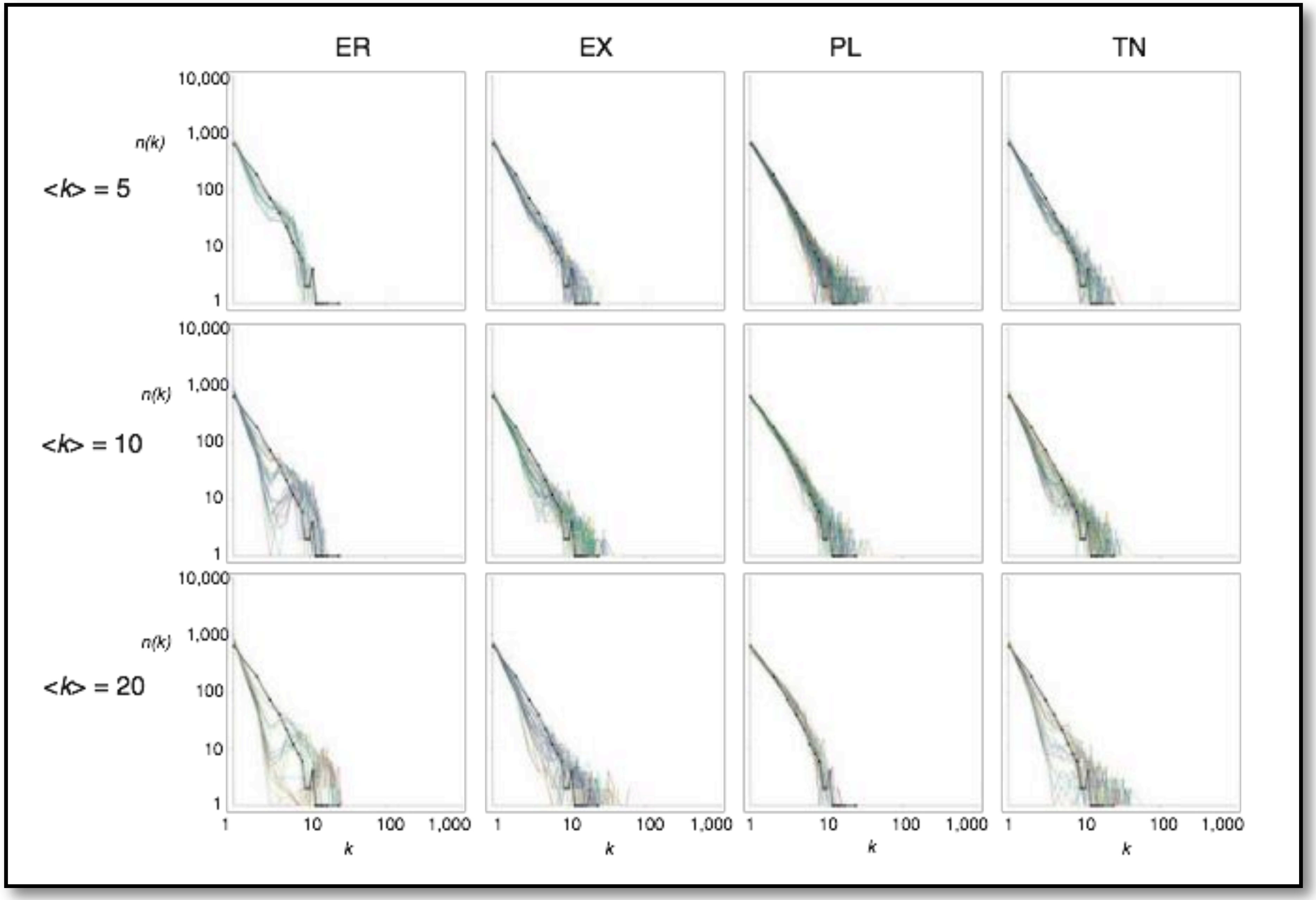
Han et al, *Nature Biotech* **23** (2005) 839

Anything Goes



Han et al, *Nature Biotech* **23** (2005) 839

Compare to Uetz et al. Data

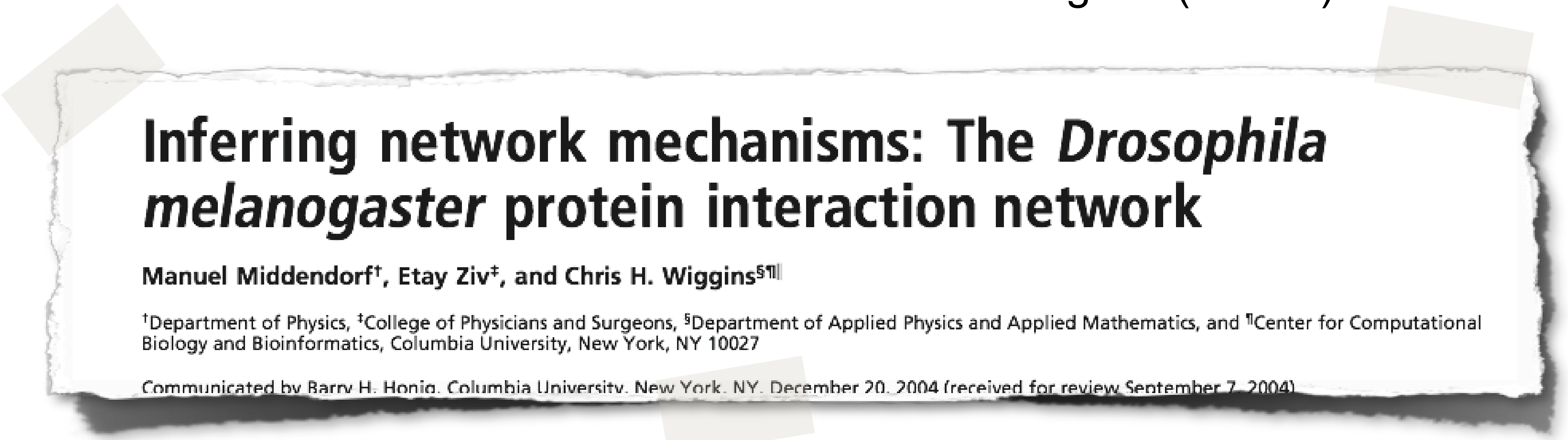


Sampling density affects observed degree distribution
→ true underlying network cannot be identified from available data

Han et al, *Nature Biotech* **23** (2005) 839

Network Growth Mechanisms

Given: an observed PPI network → how did it grow (evolve)?



Inferring network mechanisms: The *Drosophila melanogaster* protein interaction network

Manuel Middendorf[†], Etay Ziv[‡], and Chris H. Wiggins[§¶‖]

[†]Department of Physics, [‡]College of Physicians and Surgeons, [§]Department of Applied Physics and Applied Mathematics, and [¶]Center for Computational Biology and Bioinformatics, Columbia University, New York, NY 10027

Communicated by Barry H. Honig, Columbia University, New York, NY, December 20, 2004 (received for review September 7, 2004)

PNAS **102** (2005) 3192

Look at **network motifs** (local connectivity):
compare motif distributions from various network prototypes to fly network

Idea: each growth **mechanism** leads to a typical motif **distribution**, even if global measures are comparable

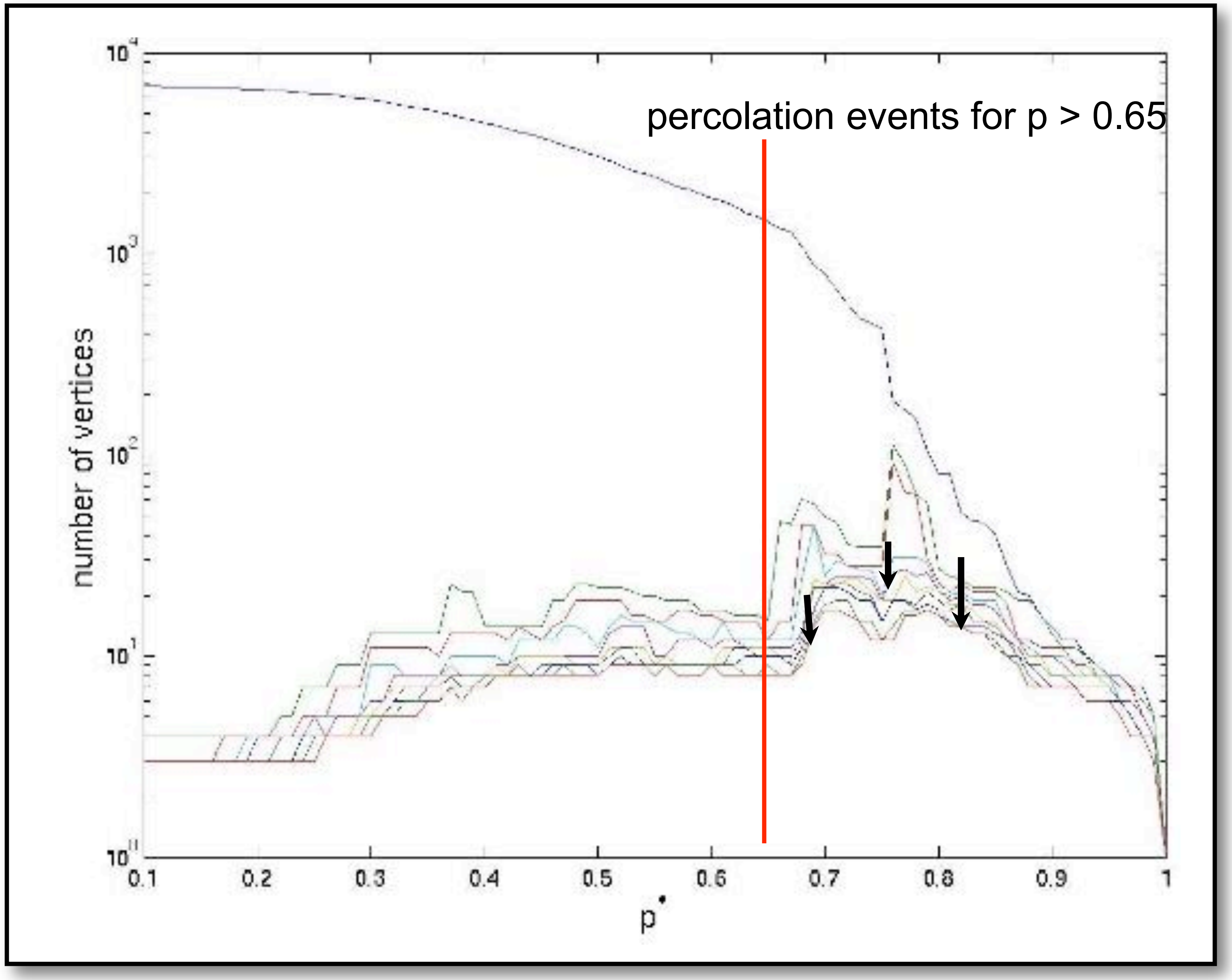
The Fly Network

Y2H PPI network for *D. melanogaster* from Giot et al. [*Science* **302** (2003) 1727]

Confidence score [0, 1] for every observed interaction
→ use only data with $p > 0.65$ (0.5)
→ remove self-interactions and isolated nodes

High confidence network with 3359 (4625) nodes and 2795 (4683) edges

Use prototype networks of same size for training

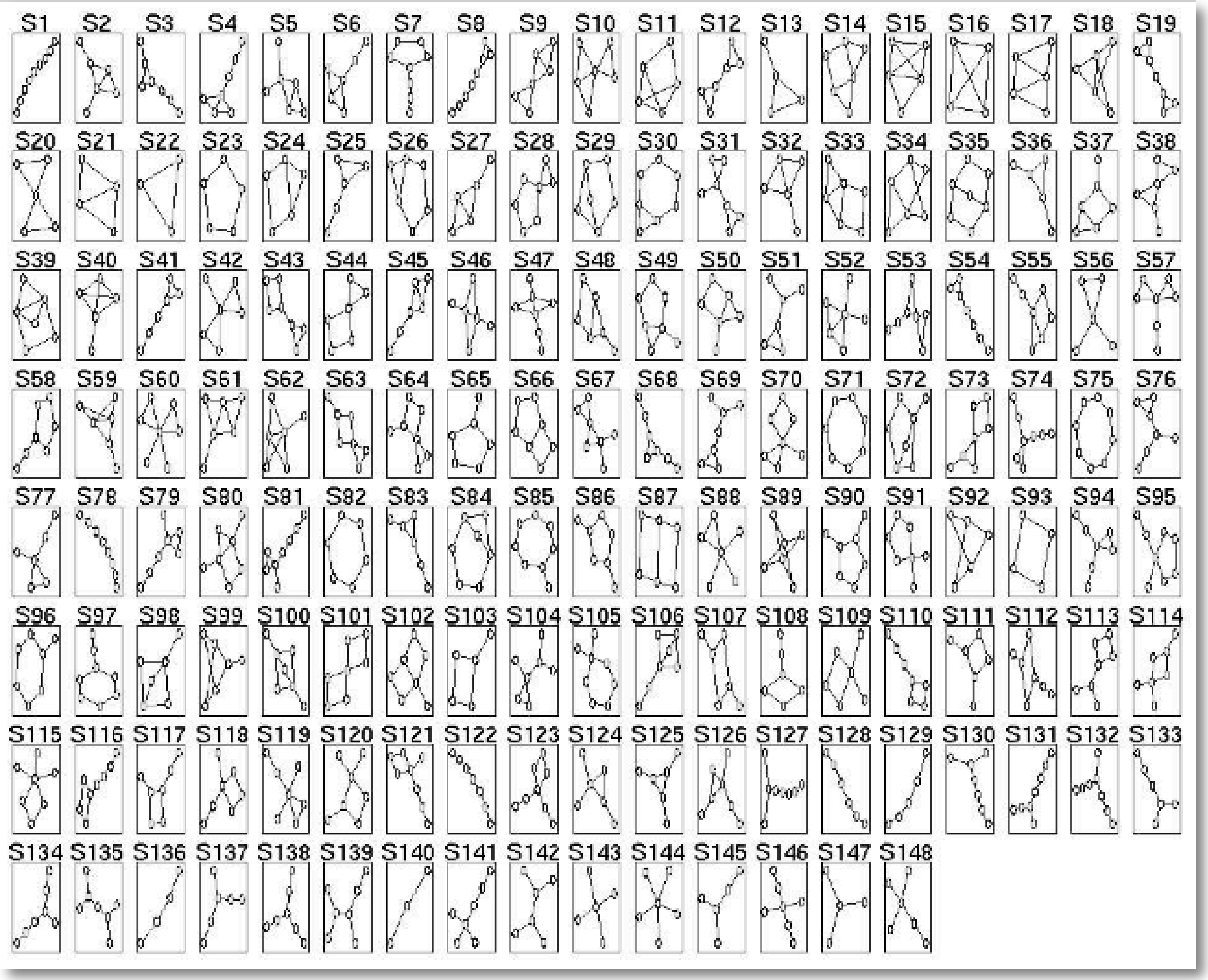


Size of largest components. At p = 0.65, there is one large component with 1433 and the other 703 components contain at most 15 nodes.

Middendorf et al, *PNAS* **102** (2005) 3192

Network Motives

All non-isomorphic subgraphs that can be generated with a walk of length 8



Middendorf et al, *PNAS* **102** (2005) 3192

Growth Mechanisms

Generate 1000 networks, each, of the following 7 types
(Same size as fly network, undefined parameters were scanned)

DMC	Duplication-mutation, preserving complementarity
DMR	Duplication with random mutations
RDS	Random static networks
RDG	Random growing network
LPA	Linear preferential attachment network
AGV	Aging vertices network
SMW	Small world network

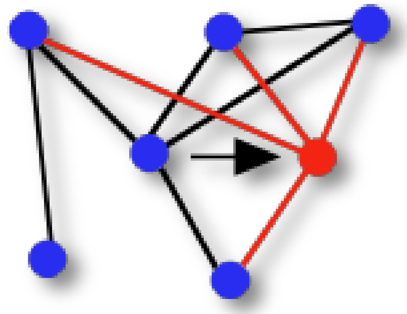
Growth Type 1: DMC

"Duplication – mutation with preserved complementarity"

Evolutionary idea: gene **duplication**, followed by a partial **loss** of function of one of the copies, making the other copy essential

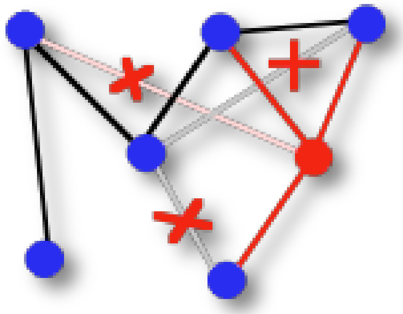
Algorithm:

Start from two connected nodes,
repeat N - 2 times:



- duplicate existing node with all interactions

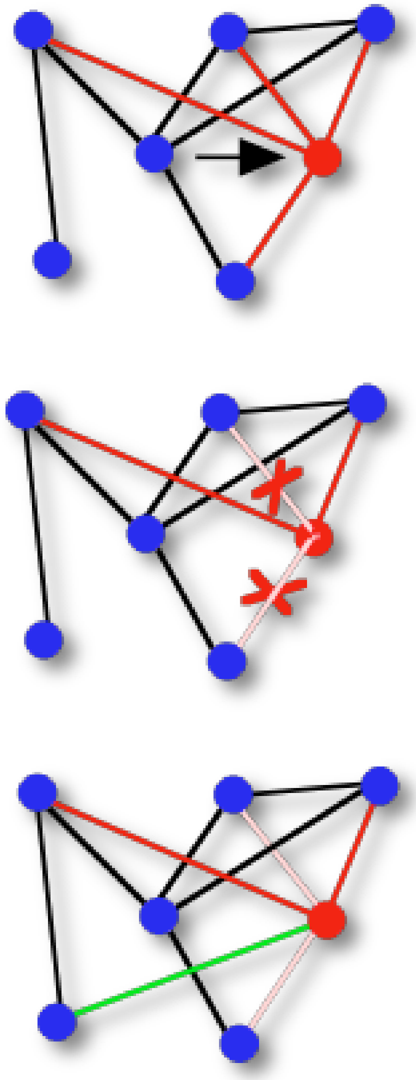
- for all neighbors: delete with probability q_{del} either link from original node **or** from copy



Growth Type 2: DMR

"Duplication with random mutations"

Gene duplication, but no correlation between original and copy (original unaffected by copy)



Algorithm:

Start from five-vertex cycle,
repeat *N* - 5 times:

- duplicate existing node with all interactions
- for all neighbors: delete with probability q_{del} link from copy
- add new links to non-neighbors with probability q_{new}/n

Growth Types 3–5: RDS, RDG, and LPA

RDS = static random network

Start from N nodes, add L links randomly

RDG = growing random network

Start from small random network, add nodes,
then edges between all existing nodes

LPA = linear preferential attachment

Add new nodes similar to Barabási-Albert algorithm,
but with preference according to (k_i + α), α = 0…5
(BA for α = 0)

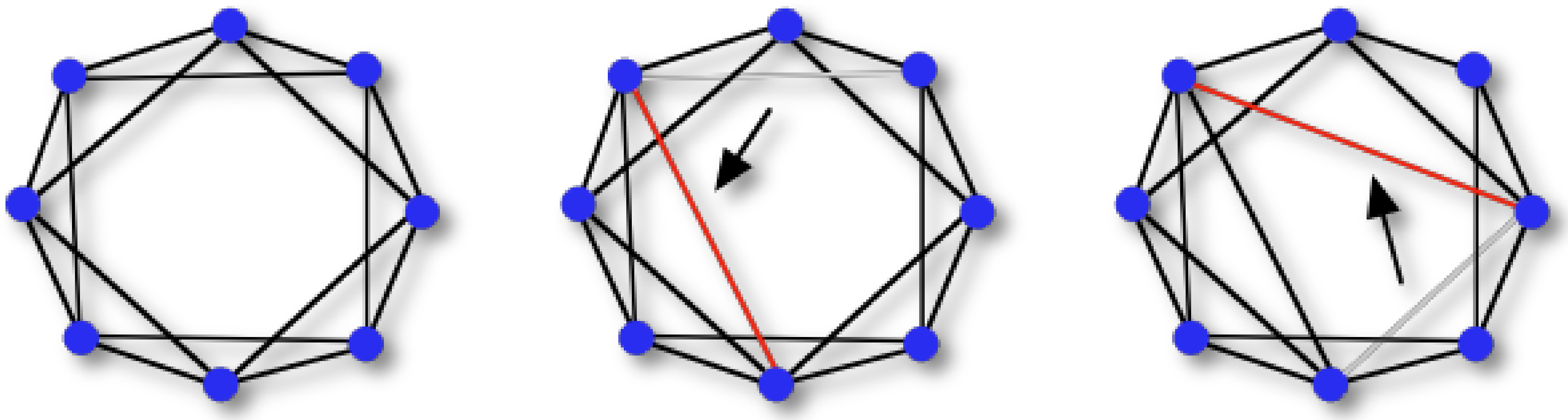
Growth Types 6-7: AGV and SMW

AGV = aging vertices network

Like growing random network,
but preference decreases with age of the node
→ citation network: more recent publications are cited more likely

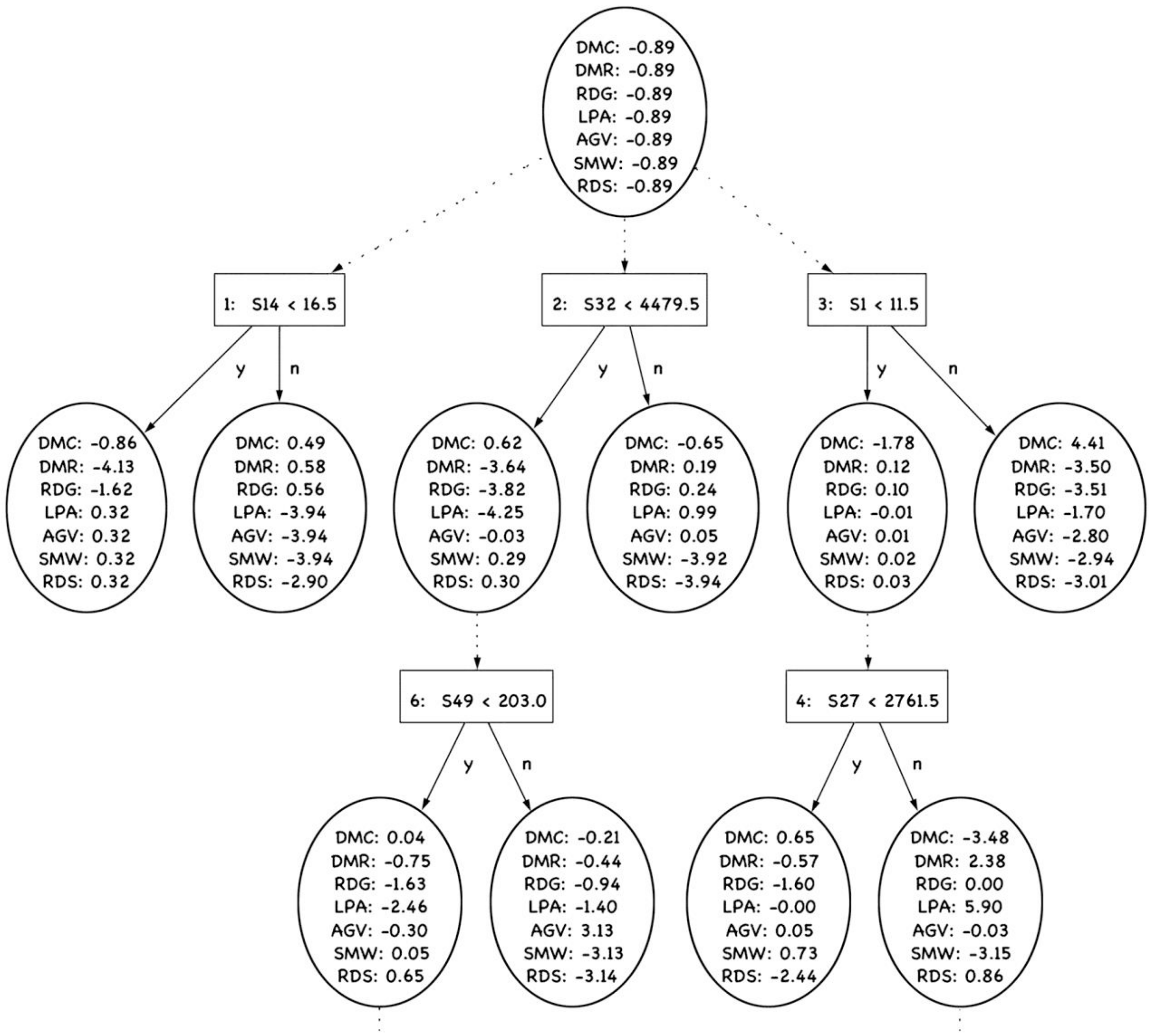
SMW = small world networks (Watts, Strogatz, *Nature* **363** (1998) 202)

Randomly rewire regular ring lattice



Alternating Decision Tree Classifier

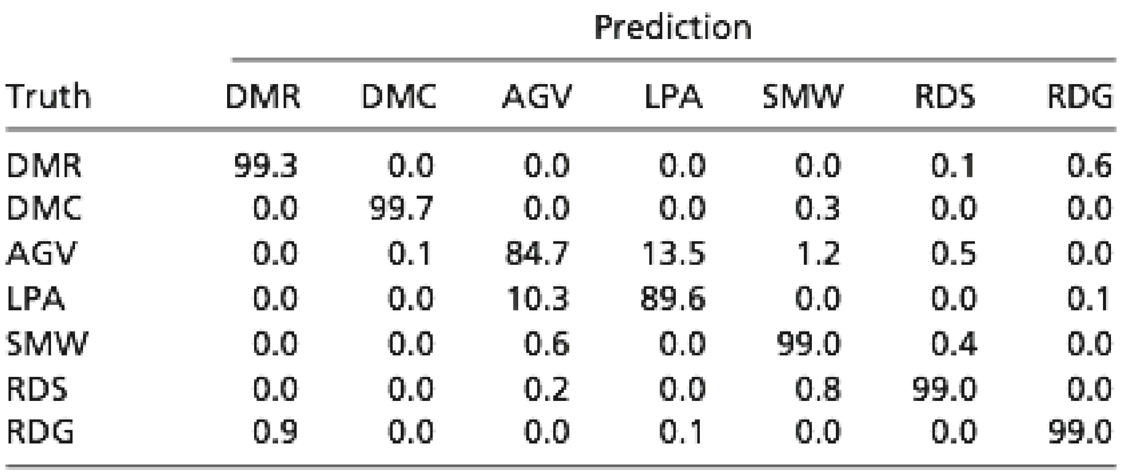
Trained with the motif counts from 1000 networks of each of the 7 types
→ prototypes are well separated and reliably classified



Part of a trained ADT

Decision nodes count
occurrence of motifs

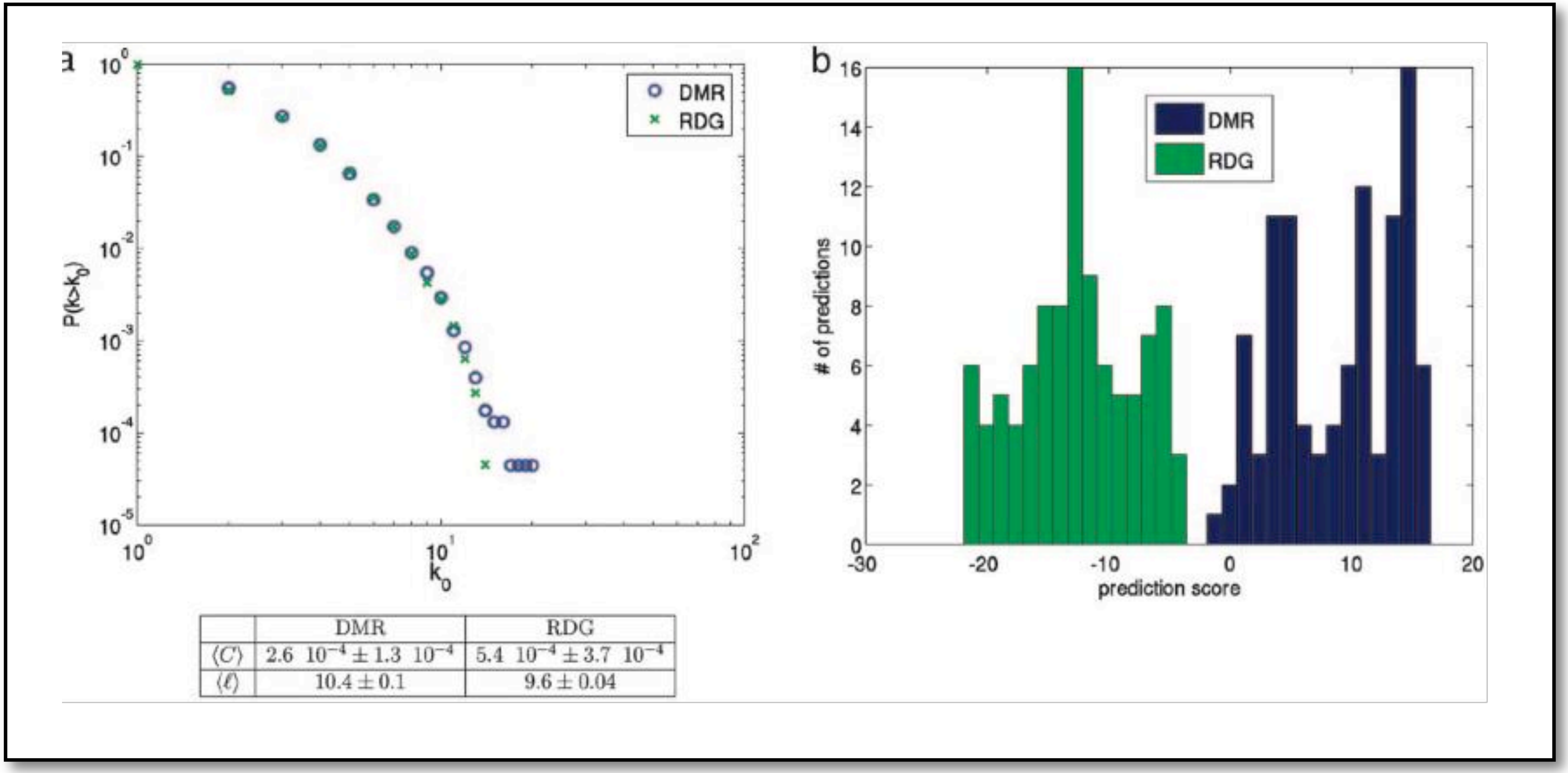
Prediction accuracy for networks similar to fly network with $p = 0.5$:



	Prediction						
Truth	DMR	DMC	AGV	LPA	SMW	RDS	RDG
DMR	99.3	0.0	0.0	0.0	0.0	0.1	0.6
DMC	0.0	99.7	0.0	0.0	0.3	0.0	0.0
AGV	0.0	0.1	84.7	13.5	1.2	0.5	0.0
LPA	0.0	0.0	10.3	89.6	0.0	0.0	0.1
SMW	0.0	0.0	0.6	0.0	99.0	0.4	0.0
RDS	0.0	0.0	0.2	0.0	0.8	99.0	0.0
RDG	0.9	0.0	0.0	0.1	0.0	0.0	99.0

Middendorf et al, *PNAS* **102** (2005) 3192

Are They Different?



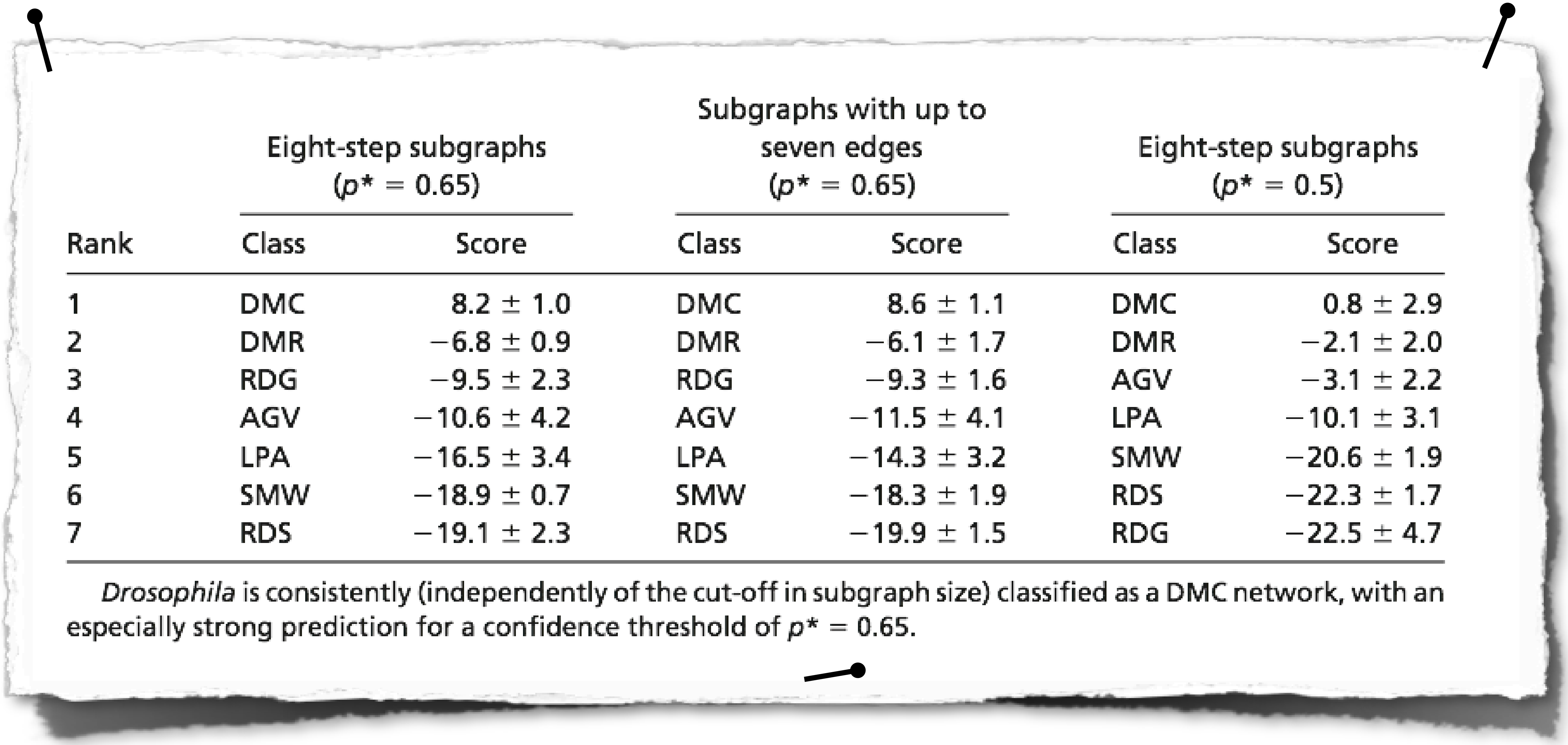
	DMR	RDG
$\langle C \rangle$	$2.6\ 10^{-4} \pm 1.3\ 10^{-4}$	$5.4\ 10^{-4} \pm 3.7\ 10^{-4}$
$\langle \ell \rangle$	10.4 ± 0.1	9.6 ± 0.04

Example DMR vs. RDG: Similar global parameters,
but different counts of the network motifs

-> networks can be perfectly separated by motif-based classifier

Middendorf et al, *PNAS* **102** (2005) 3192

How Did the Fly Evolve?



	Eight-step subgraphs ($p^* = 0.65$)		Subgraphs with up to seven edges ($p^* = 0.65$)		Eight-step subgraphs ($p^* = 0.5$)	
Rank	Class	Score	Class	Score	Class	Score
1	DMC	8.2 ± 1.0	DMC	8.6 ± 1.1	DMC	0.8 ± 2.9
2	DMR	−6.8 ± 0.9	DMR	−6.1 ± 1.7	DMR	−2.1 ± 2.0
3	RDG	−9.5 ± 2.3	RDG	−9.3 ± 1.6	AGV	−3.1 ± 2.2
4	AGV	−10.6 ± 4.2	AGV	−11.5 ± 4.1	LPA	−10.1 ± 3.1
5	LPA	−16.5 ± 3.4	LPA	−14.3 ± 3.2	SMW	−20.6 ± 1.9
6	SMW	−18.9 ± 0.7	SMW	−18.3 ± 1.9	RDS	−22.3 ± 1.7
7	RDS	−19.1 ± 2.3	RDS	−19.9 ± 1.5	RDG	−22.5 ± 4.7

Drosophila is consistently (independently of the cut-off in subgraph size) classified as a DMC network, with an especially strong prediction for a confidence threshold of $p^* = 0.65$.

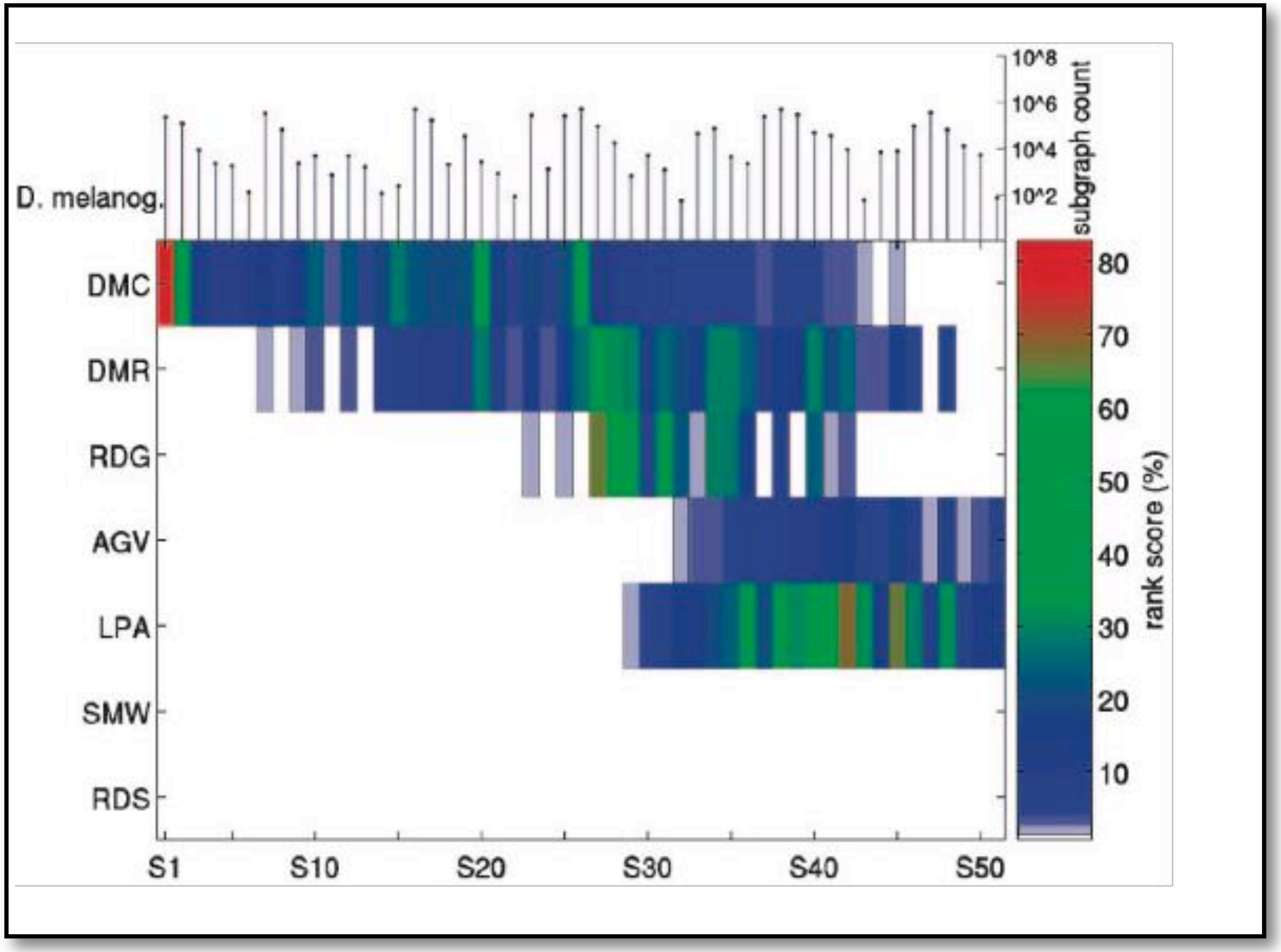
→ Best overlap with DMC (Duplication-mutation, preserved complementarity)

→ Scale-free or random networks are very unlikely

→ what about protein-domain-interaction network of Thomas et al?

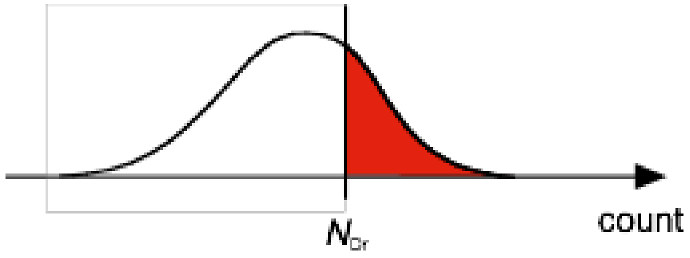
Middendorf et al, *PNAS* **102** (2005) 3192

Motif Count Frequencies



-> DMC and DMR networks contain most subgraphs in similar amount as fly network.

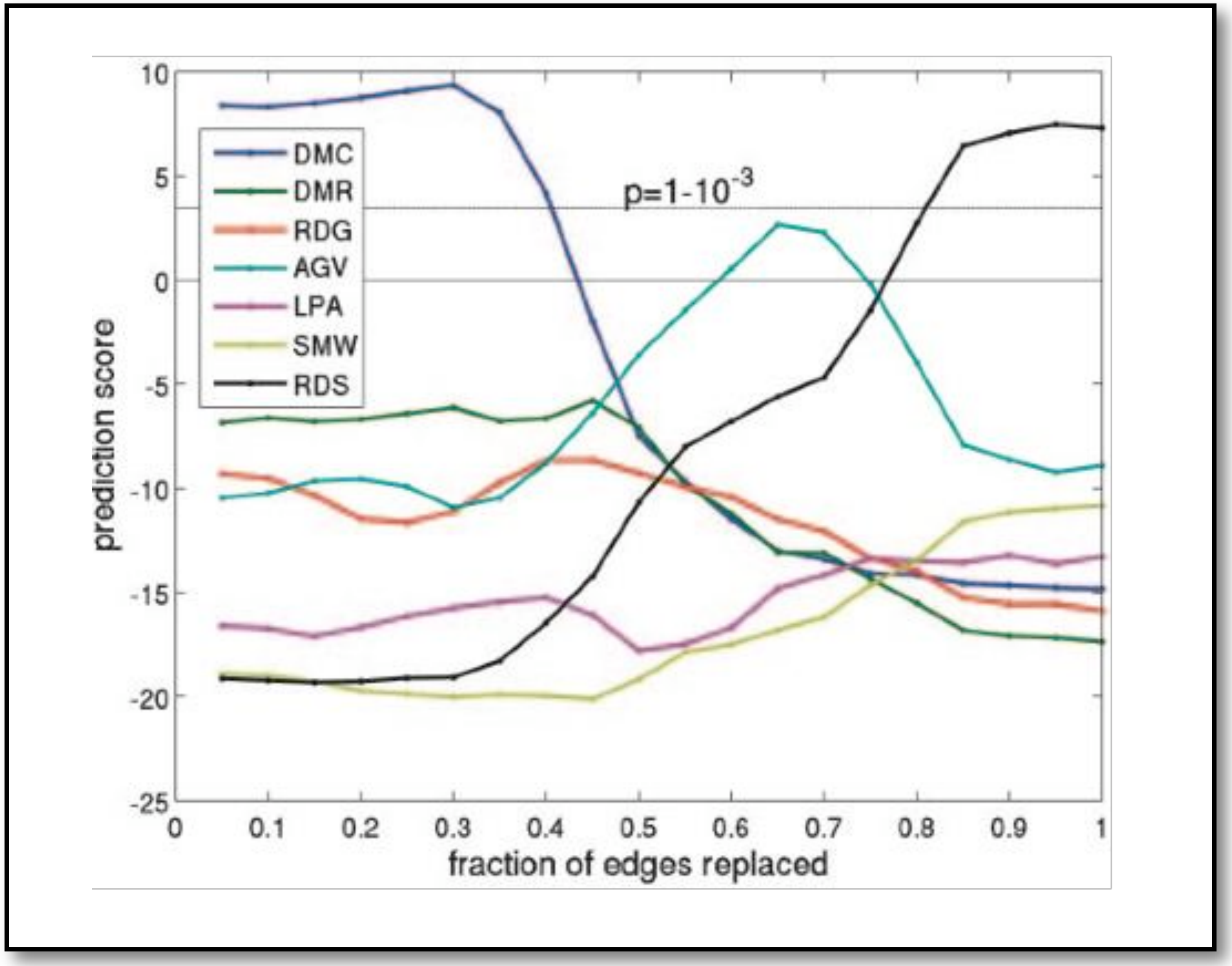
rank score: fraction of test networks with a higher count than Drosophila (50% = same count as fly on avg.)



Middendorf et al, *PNAS* **102** (2005) 3192

Experimental Errors?

Randomly replace edges in **fly** network and **classify** again:



→ Classification **unchanged** for **≤ 30%** incorrect edges

Summary (I)

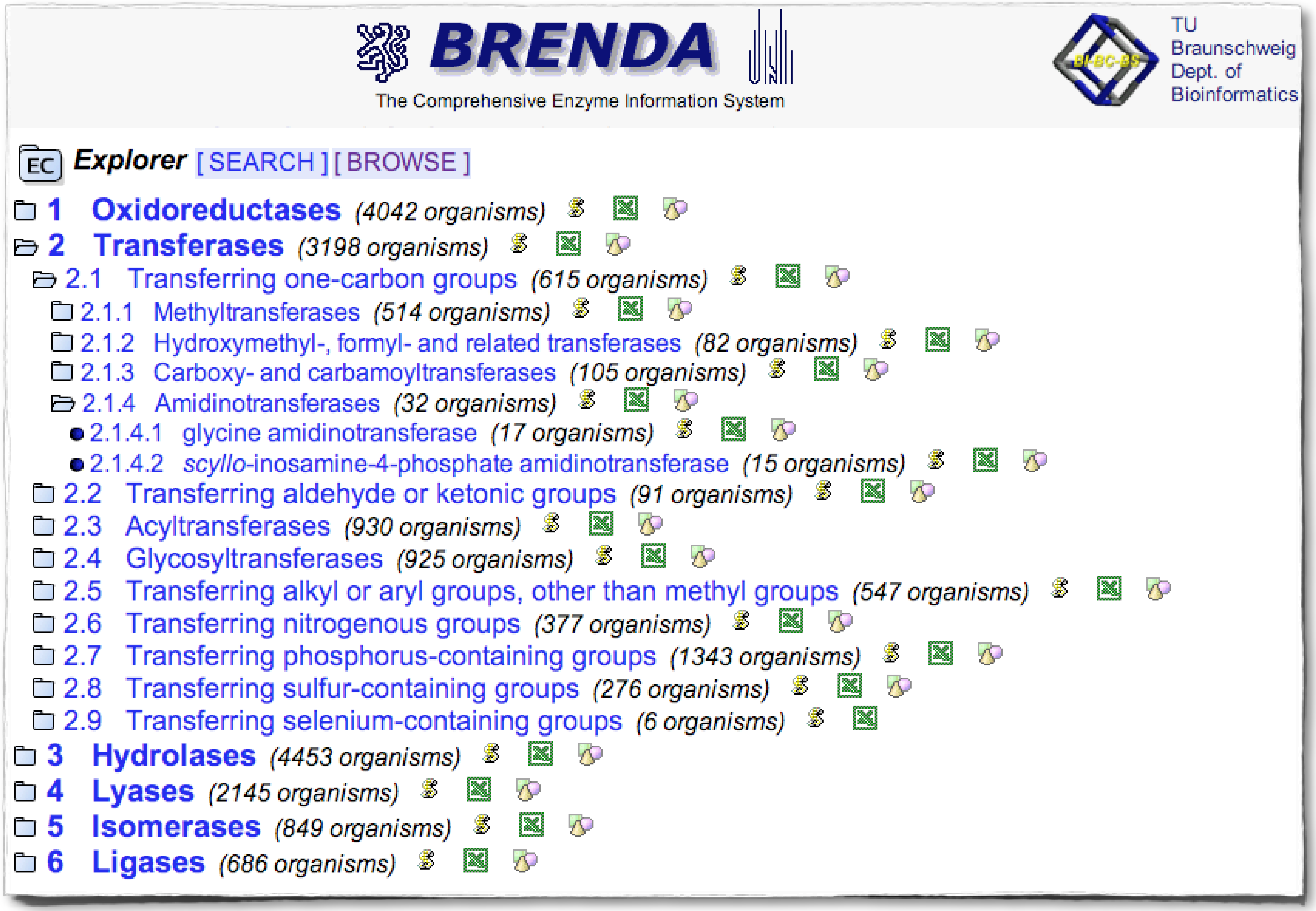
Sampling matters!

→ "Scale-free" *P(k)* obtained by sparse sampling from many network types

Test different **hypotheses** for

- **global** features
  - → depends on unknown parameters and sampling
    - → no clear statement possible
- **local** features (motifs)
  - → are better preserved
    - → DMC best among tested prototypes

What Does a Protein Do?

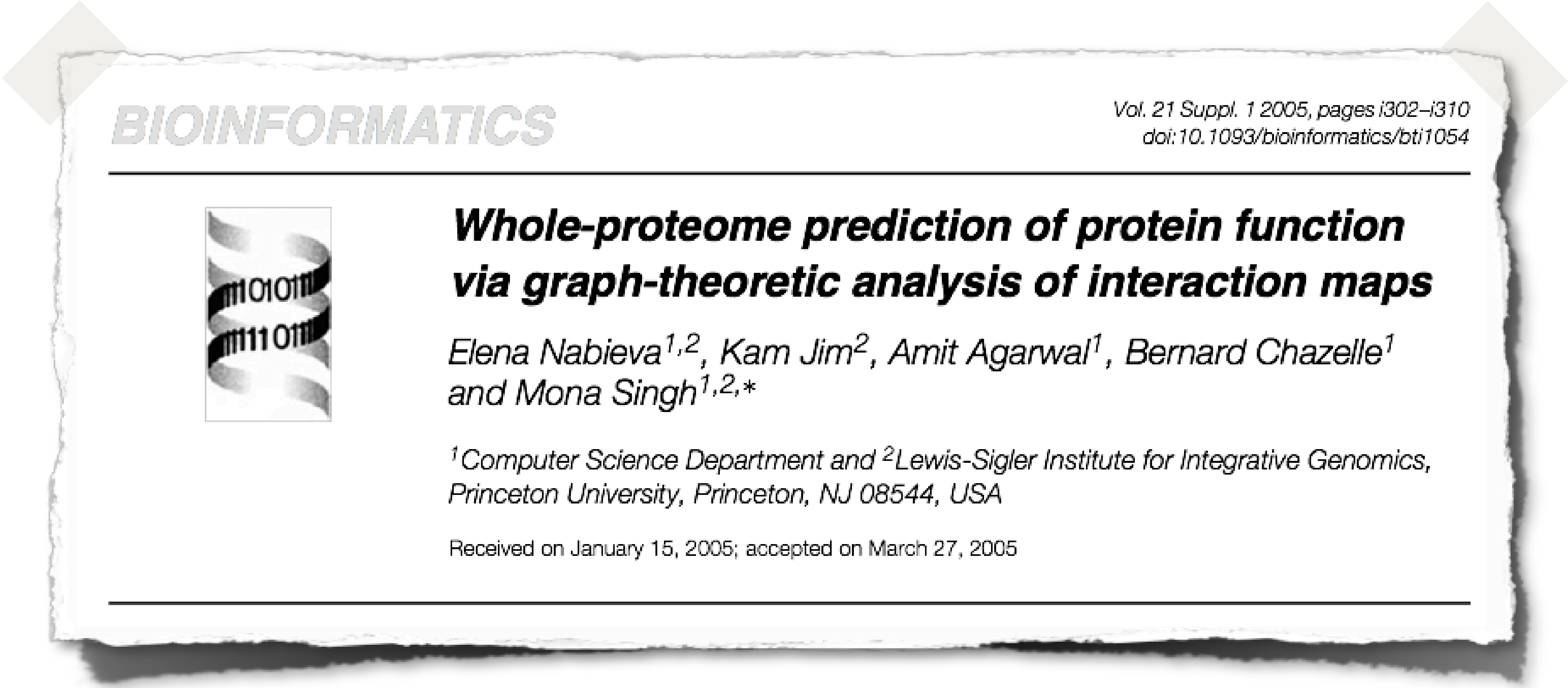


Explorer [SEARCH] [BROWSE]

- **1 Oxidoreductases** *(4042 organisms)*
- **2 Transferases** *(3198 organisms)*
  - 2.1 Transferring one-carbon groups *(615 organisms)*
    - 2.1.1 Methyltransferases *(514 organisms)*
    - 2.1.2 Hydroxymethyl-, formyl- and related transferases *(82 organisms)*
    - 2.1.3 Carboxy- and carbamoyltransferases *(105 organisms)*
    - 2.1.4 Amidinotransferases *(32 organisms)*
      - 2.1.4.1 glycine amidinotransferase *(17 organisms)*
      - 2.1.4.2 *scyllo*-inosamine-4-phosphate amidinotransferase *(15 organisms)*
  - 2.2 Transferring aldehyde or ketonic groups *(91 organisms)*
  - 2.3 Acyltransferases *(930 organisms)*
  - 2.4 Glycosyltransferases *(925 organisms)*
  - 2.5 Transferring alkyl or aryl groups, other than methyl groups *(547 organisms)*
  - 2.6 Transferring nitrogenous groups *(377 organisms)*
  - 2.7 Transferring phosphorus-containing groups *(1343 organisms)*
  - 2.8 Transferring sulfur-containing groups *(276 organisms)*
  - 2.9 Transferring selenium-containing groups *(6 organisms)*
- **3 Hydrolases** *(4453 organisms)*
- **4 Lyases** *(2145 organisms)*
- **5 Isomerases** *(849 organisms)*
- **6 Ligases** *(686 organisms)*

Enzyme **C**lassification scheme
(from http://www.brenda-enzymes.org/)

Un-Classified Proteins?



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doi:10.1093/bioinformatics/bti1054

Whole-proteome prediction of protein function via graph-theoretic analysis of interaction maps

Elena Nabieva[1,2], Kam Jim[2], Amit Agarwal[1], Bernard Chazelle[1] and Mona Singh[1,2,*]

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Received on January 15, 2005; accepted on March 27, 2005

Many **unclassified proteins**:

→ estimate: ~1/3 of the yeast proteome not annotated functionally

→ BioGRID: 4495 proteins in the largest cluster of the yeast physical interaction map.

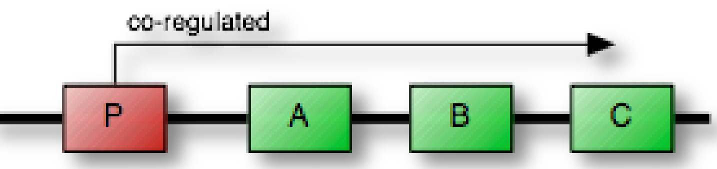
2946 have a MIPS functional annotation

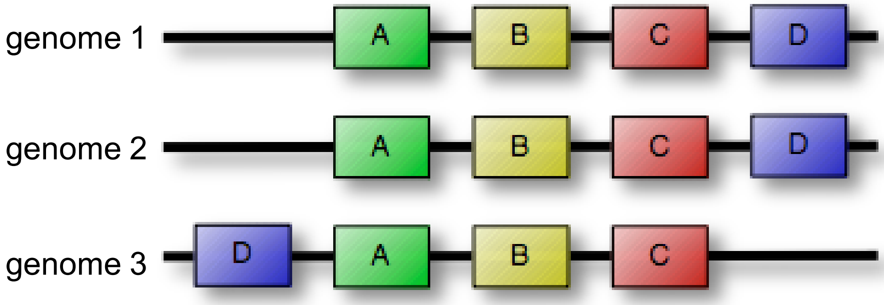
Partition the Graph

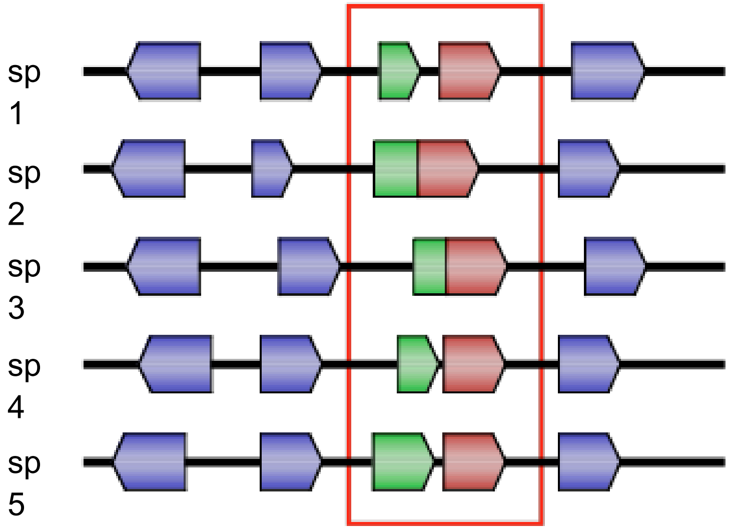
Large **PPI networks** were built from:

- HT experiments (Y2H, TAP, synthetic lethality, coexpression, coregulation, …)
- predictions (gene profiling, gene neighborhood, phylogenetic profiles, …)

→ proteins that are functionally linked





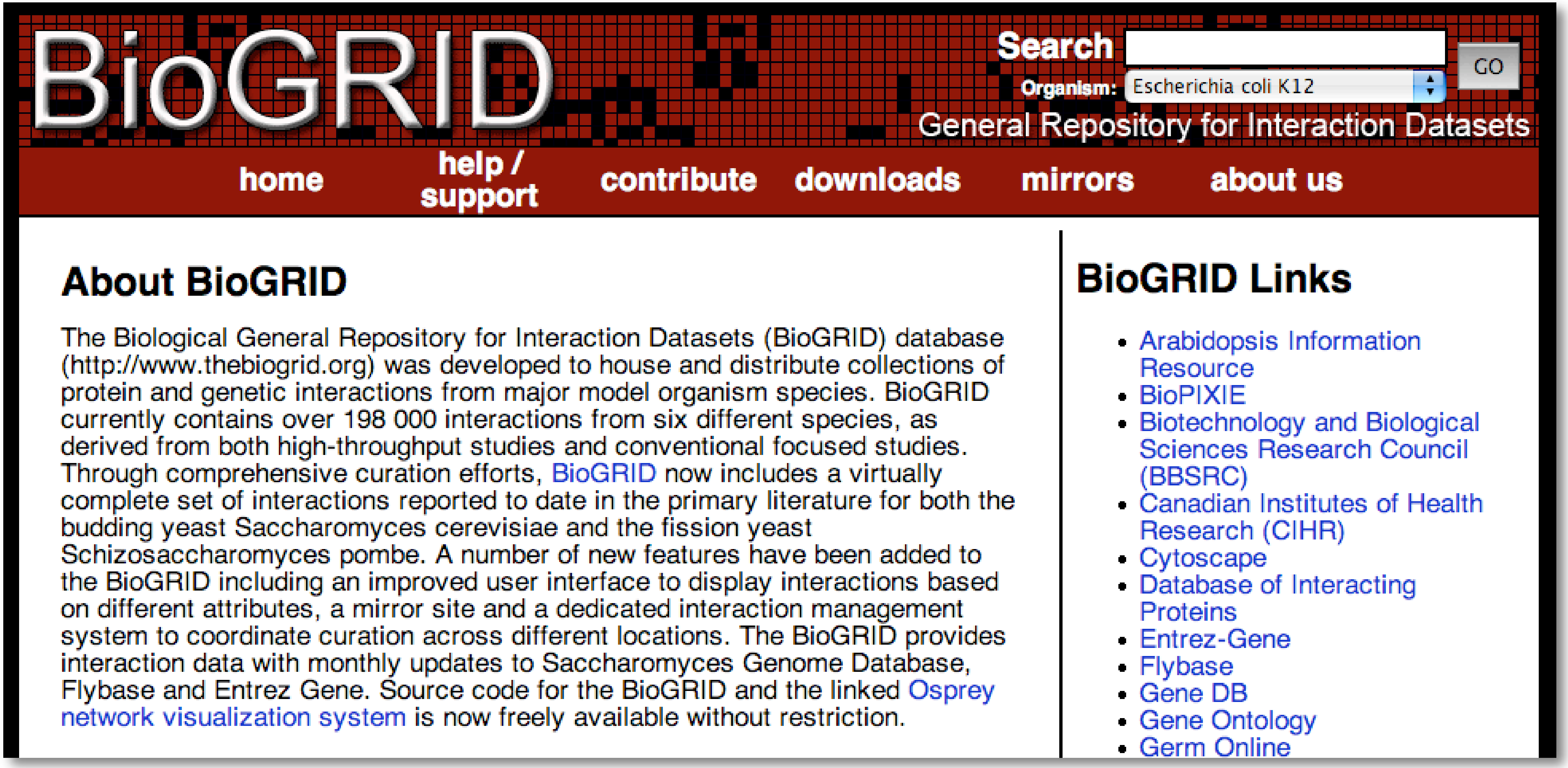


Identify **unknown functions** from **clustering** of these networks by, e.g.:

- shared interactions (similar neighborhood → power graphs)
- membership in a community
- similarity of shortest path vectors to all other proteins (= similar path into the rest of the network)

Protein Interactions

Nabieva et al used the *S. cerevisiae* dataset from GRID of 2005 (now BioGRID)
→ 4495 proteins and 12 531 physical interactions in the largest cluster



BioGRID
Search
GO
Organism: Escherichia coli K12
General Repository for Interaction Datasets
home
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downloads
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About BioGRID

The Biological General Repository for Interaction Datasets (BioGRID) database (http://www.thebiogrid.org) was developed to house and distribute collections of protein and genetic interactions from major model organism species. BioGRID currently contains over 198 000 interactions from six different species, as derived from both high-throughput studies and conventional focused studies. Through comprehensive curation efforts, BioGRID now includes a virtually complete set of interactions reported to date in the primary literature for both the budding yeast Saccharomyces cerevisiae and the fission yeast Schizosaccharomyces pombe. A number of new features have been added to the BioGRID including an improved user interface to display interactions based on different attributes, a mirror site and a dedicated interaction management system to coordinate curation across different locations. The BioGRID provides interaction data with monthly updates to Saccharomyces Genome Database, Flybase and Entrez Gene. Source code for the BioGRID and the linked Osprey network visualization system is now freely available without restriction.

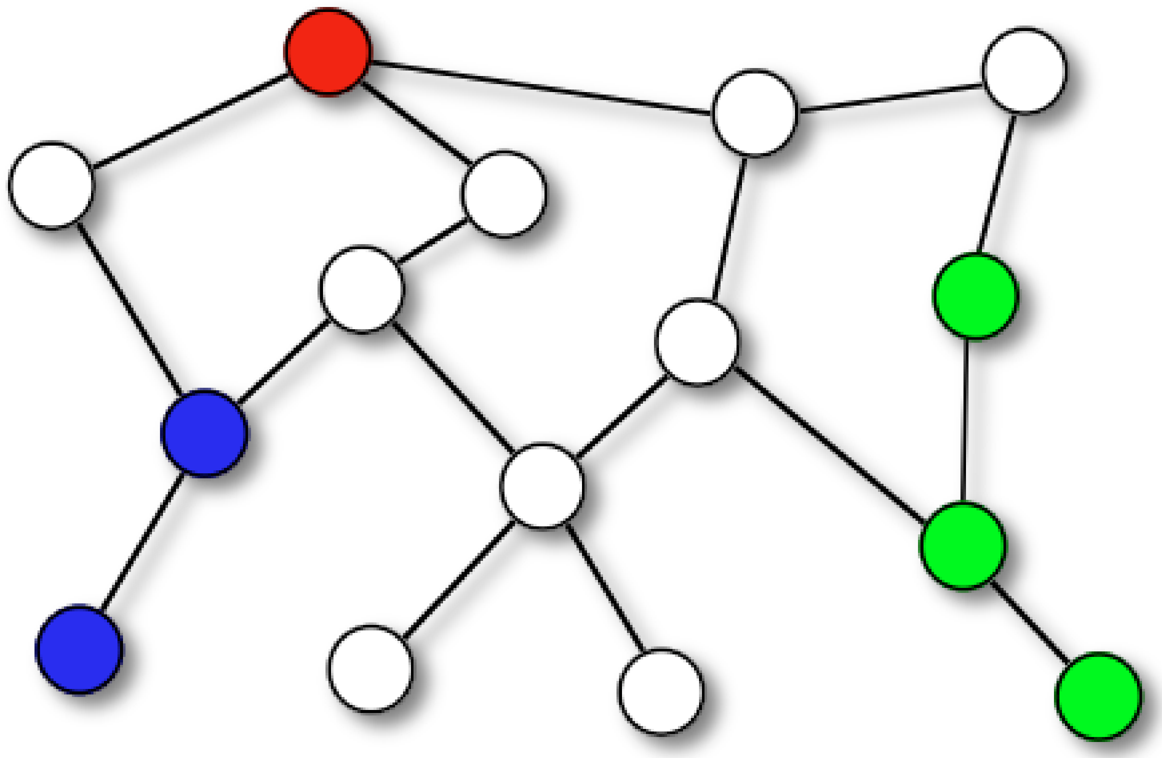
BioGRID Links

- Arabidopsis Information Resource
- BioPIXIE
- Biotechnology and Biological Sciences Research Council (BBSRC)
- Canadian Institutes of Health Research (CIHR)
- Cytoscape
- Database of Interacting Proteins
- Entrez-Gene
- Flybase
- Gene DB
- Gene Ontology
- Germ Online

http://www.thebiogrid.org/about.php

Function Annotation

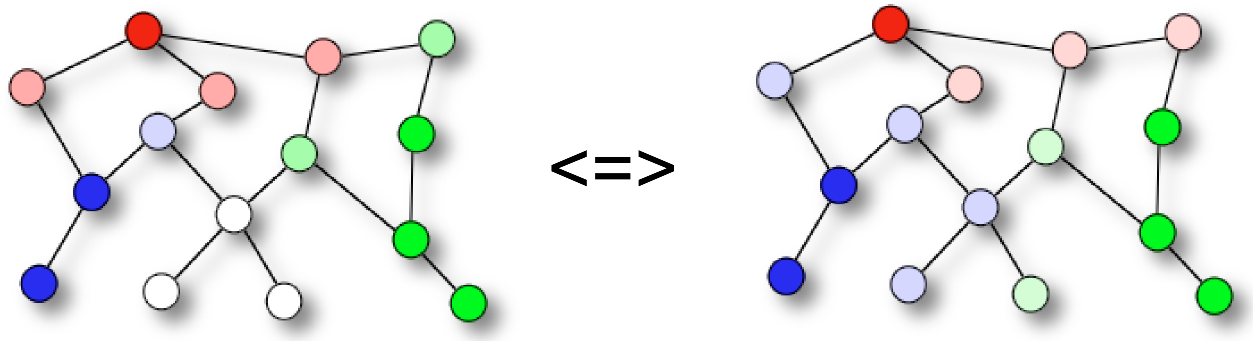
Task: **predict** function (= functional annotation) for a protein from the **available** annotations



Similar:
How to **assign colors** to the white nodes?

Use information on:

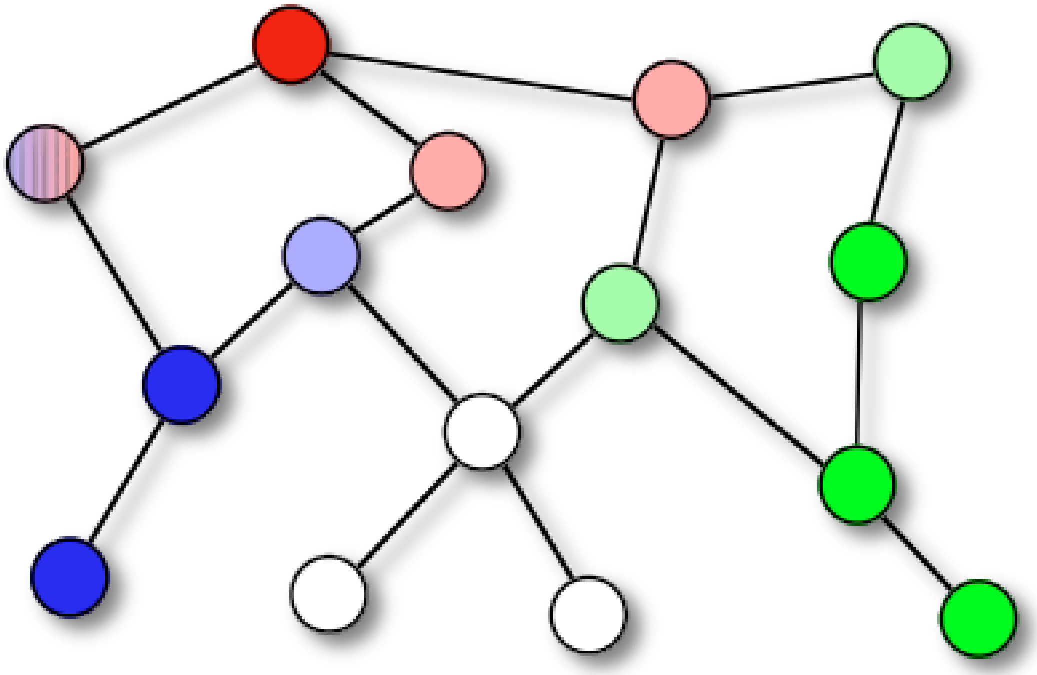
- distance to colored nodes
- local connectivity
- reliability of the links
- …



Algorithm I: Majority

Schwikowski, Uetz, and Fields, " A network of protein–protein interactions in yeast" *Nat. Biotechnol.* **18** (2000) 1257

Consider all neighbors and **sum** up how often a certain **annotation occurs**
→ score for an annotation = count among the direct neighbors
→ take the 3 most frequent functions



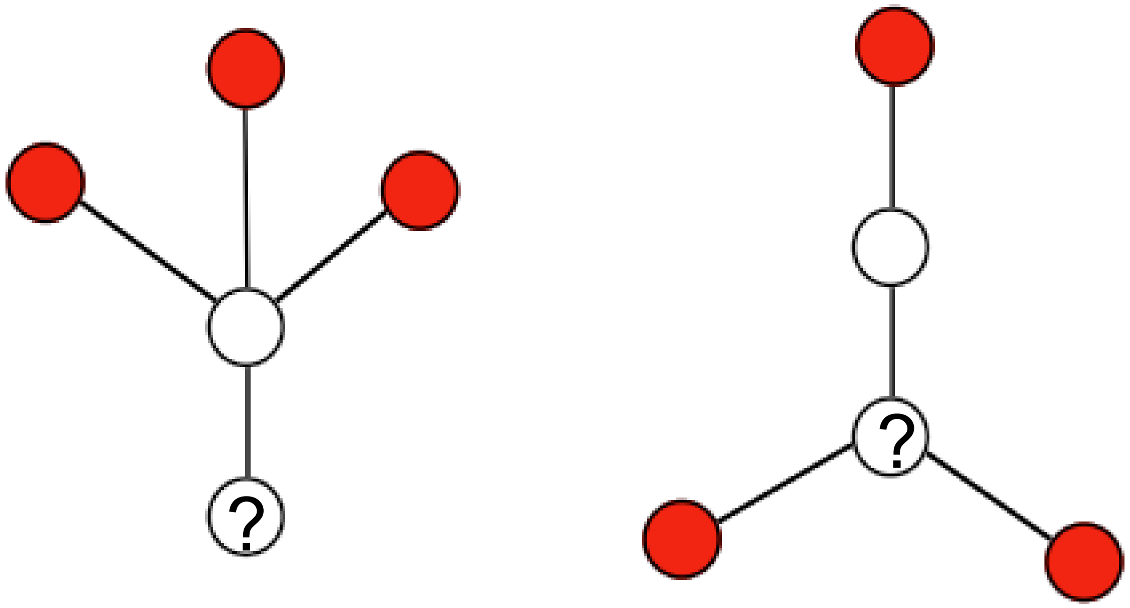
Majority makes only limited use of the local connectivity
→ cannot assign function to next-neighbors

For weighted graphs:
→ weighted sum

Extended Majority: Neighborhood

Hishigaki, Nakai, Ono, Tanigami, and Takagi, "Assessment of prediction accuracy of protein function from protein–protein interaction data", *Yeast* **18** (2001) 523

Look for **overrepresented** functions within a given **radius** of 1, 2, or 3 links → use as function score the value of a χ^2–test



Neighborhood does not consider local network topology

Both examples are treated **identically** with $r = 2$

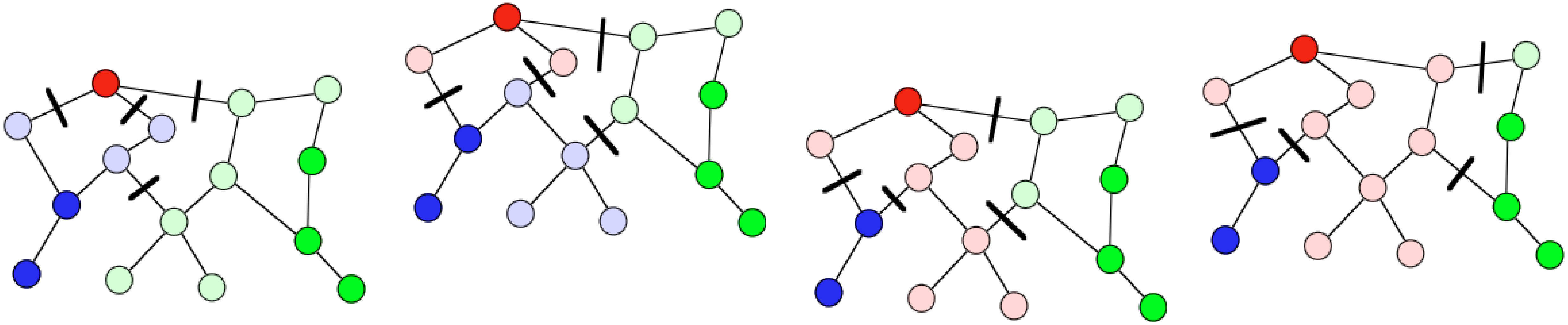
Neighborhood can not (easily) be generalized to weighted graphs!

Minimize Changes: GenMultiCut

Karaoz, Murali, Letovsky, Zheng, Ding, Cantor, and Kasif, "Whole-genome annotation by using evidence integration in functional-linkage networks" PNAS **101** (2004) 2888

"Annotate proteins so as to **minimize** the number of times that **different** functions are associated with **neighboring** proteins"

→ generalization of the multiway *k*-cut problem for weighted edges, can be stated as an integer linear program (ILP)



Multiple possible solutions → scores from **frequency** of annotations

Nabieva *et al*: FunctionalFlow

Extend the idea of **"guilty by association"**

→ each annotated protein is a source of "function"-flow

→ simulate for a few time steps

→ choose the annotation *a* with the highest accumulated flow

Each node *u* has a reservoir $R_t(u)$, each edge a capacity constraint (weight) $w_{u,v}$

Initially: $R_0^a(u) = \begin{cases} \infty, & \text{if } u \text{ is annotated with } a, \\ 0, & \text{otherwise.} \end{cases}$ and $g_0^a(u,v) = 0$

Then: **downhill flow** with capacity constraints

$$g_t^a(u,v) = \begin{cases} 0, & \text{if } R_{t-1}^a(u) < R_{t-1}^a(v) \\ \min\left(w_{u,v}, \frac{w_{u,v}}{\sum_{(u,y)\in E} w_{u,y}}\right), & \text{otherwise.} \end{cases}$$

Score from accumulated in-flow: $f_a(u) = \sum_{t=1}^{d} \sum_{v:(u,v)\in E} g_t^a(v,u)$

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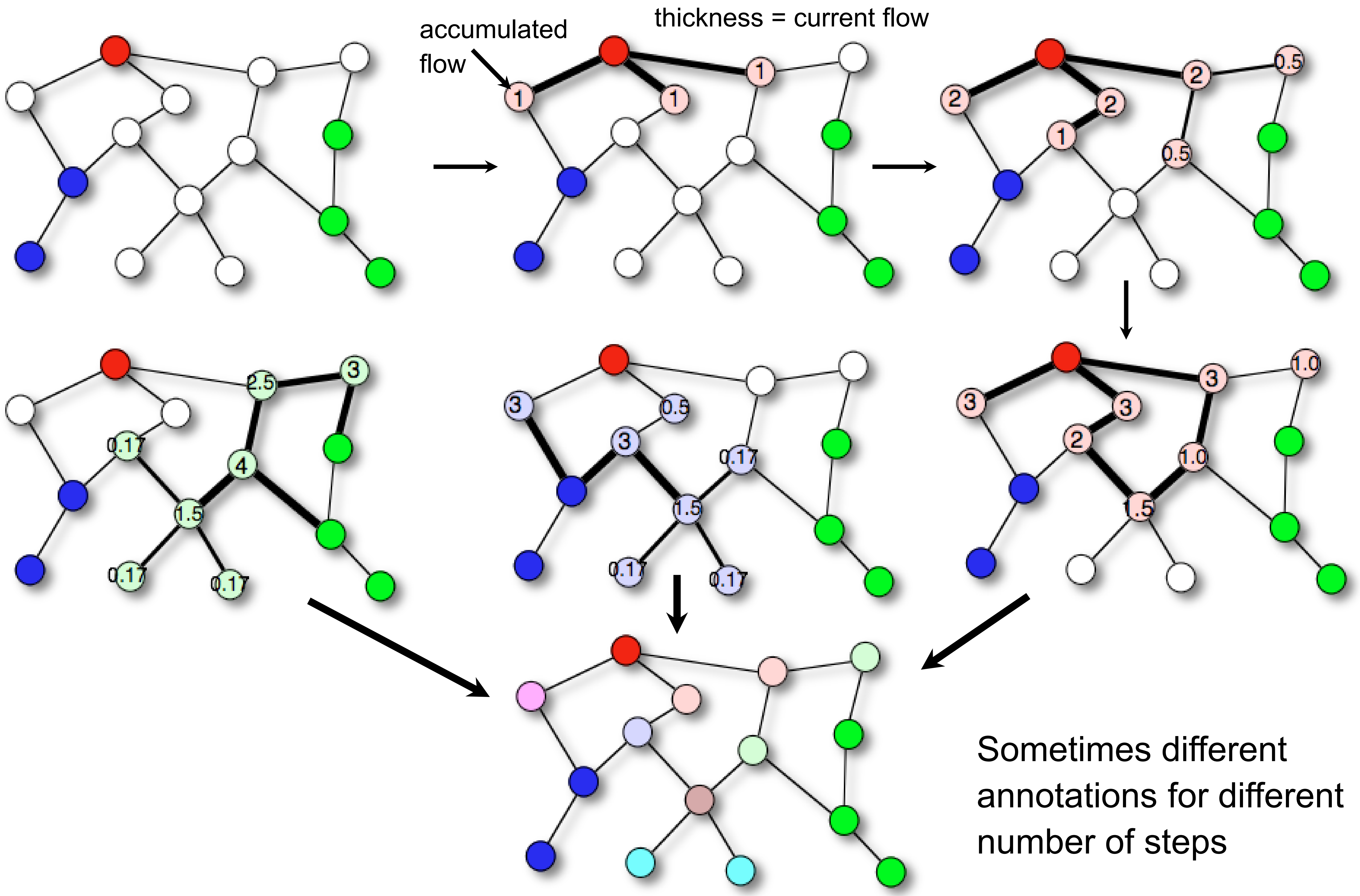
An Example

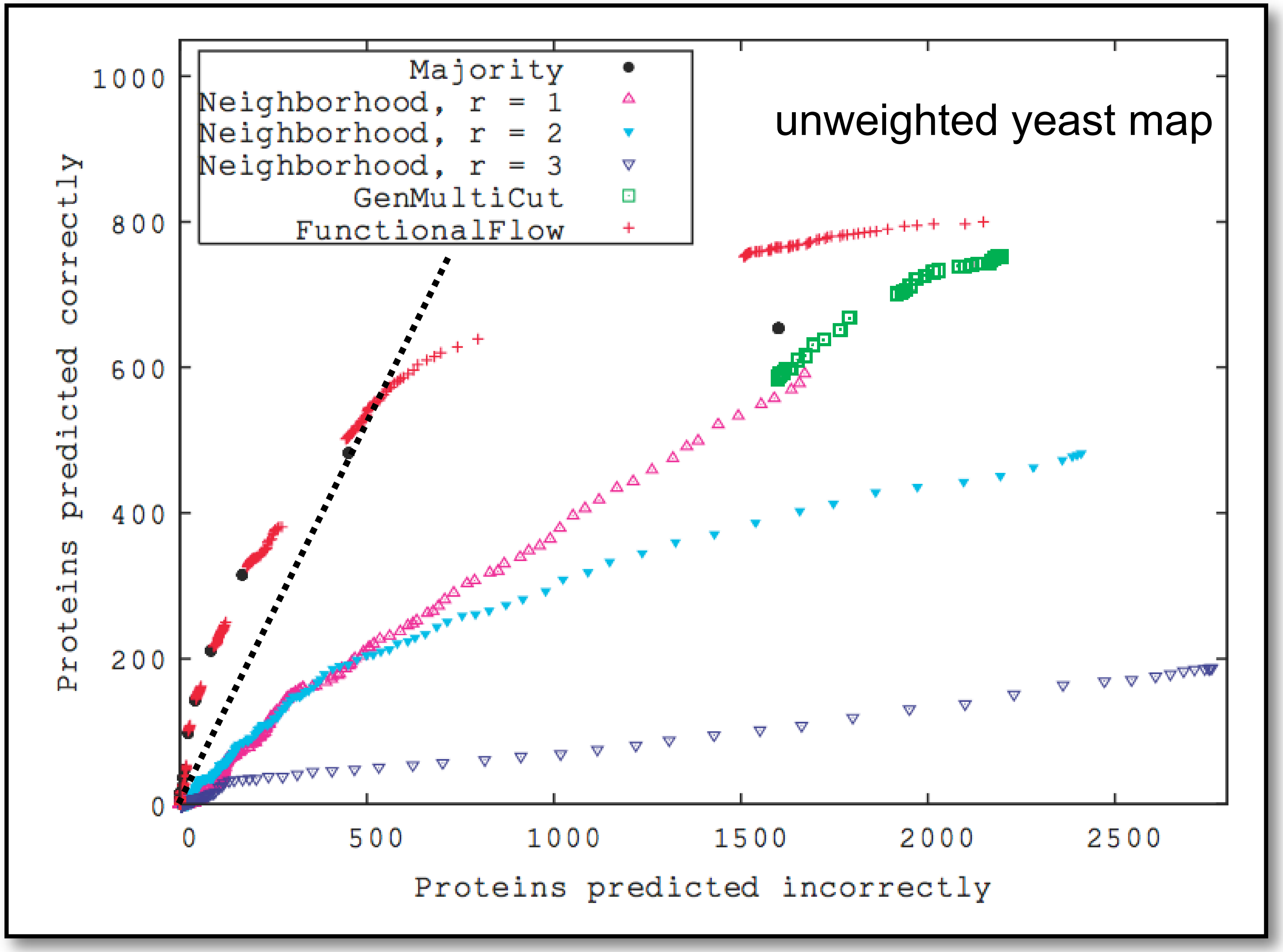


accumulated flow

thickness = current flow

Sometimes different annotations for different number of steps

Comparison



For FunctionalFlow: six propagation steps (diameter of the yeast network ≈ 12)

Change **score threshold** for accepting annotations → ratio **TP/FP**

→ **FunctionalFlow** performs **best** in the high-confidence region

→ many false predictions!!!

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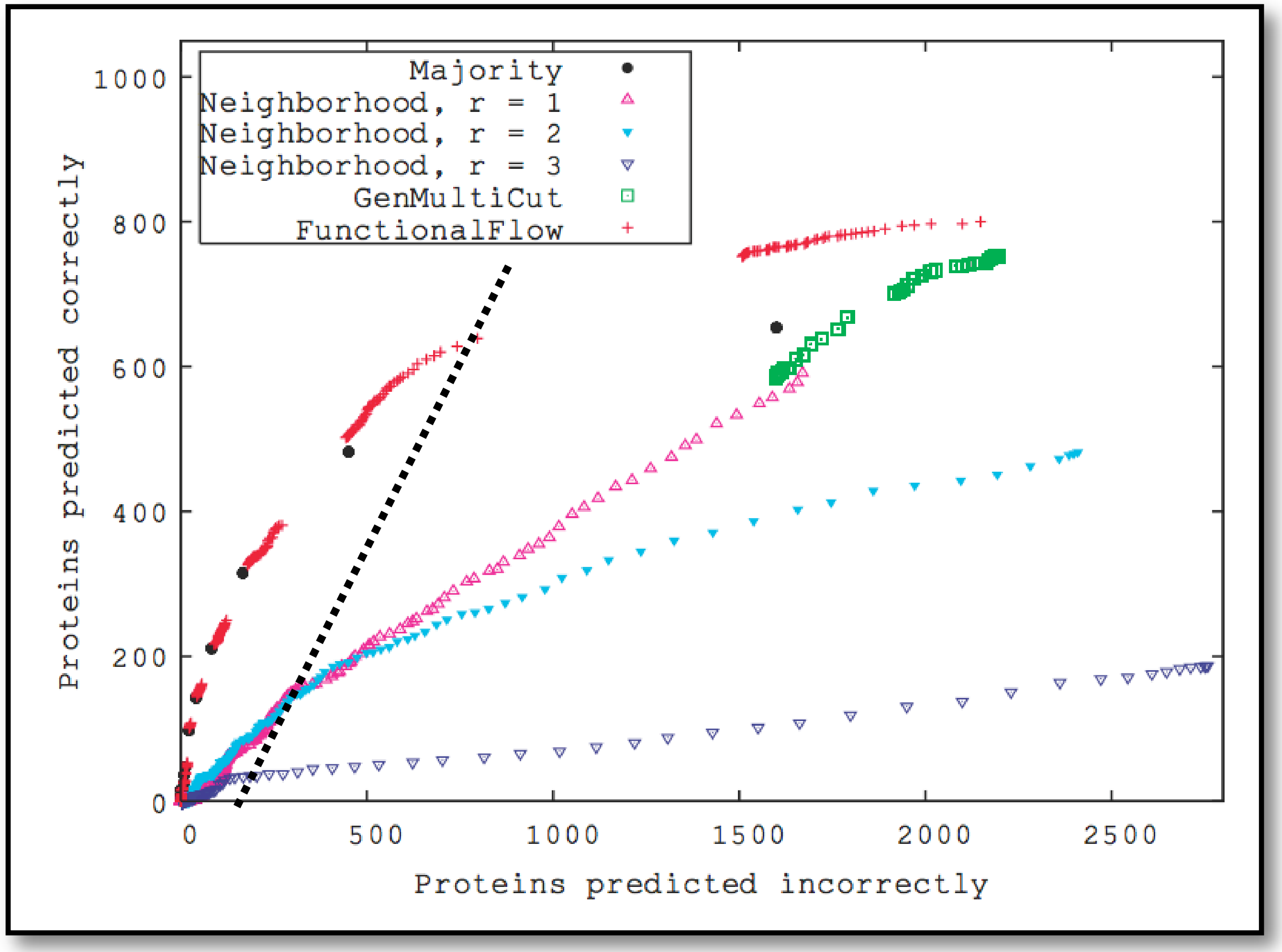
Comparison Details



Multiple runs (solutions) of FunctionalFlow
(with slight random perturbations of the weights)
→ increases prediction accuracy

Majority vs. Neighborhood @ *r* = 1
→ counting neighboring annotations is more effective than χ^2-test

Neighborhood with *r* = 1 comparable to FunctionalFlow
for high-confidence region, performance decreases with increasing *r*
→ **bad** idea to **ignore** local connectivity

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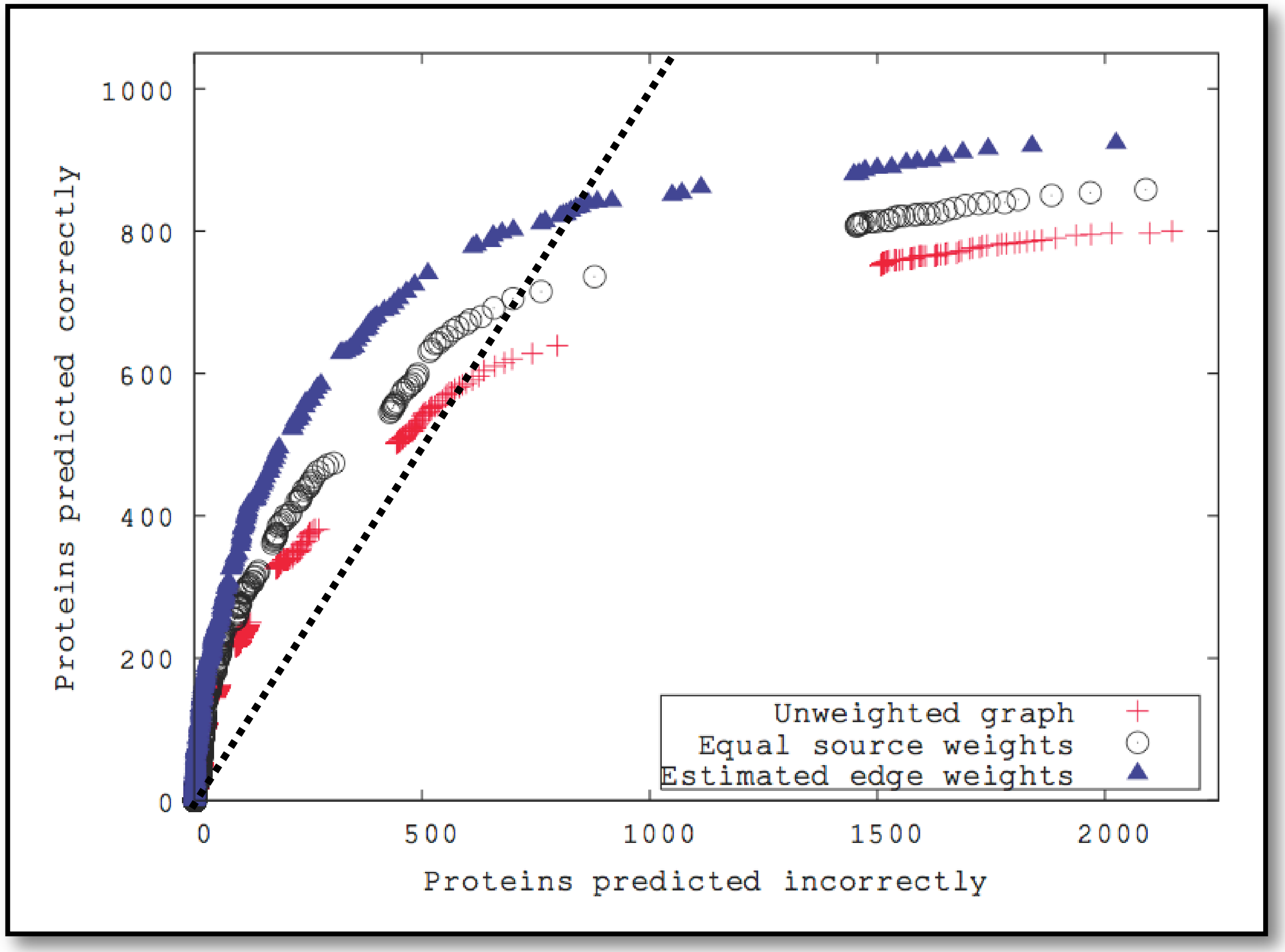
Weighted Graphs

Performance of FunctionalFlow with differently weighted data:



Compare:

- unweighted
- weight 0.5 per experiment
- weight for experiments according to (estimated) reliability

Largest improvement
→ individual experimental reliabilities

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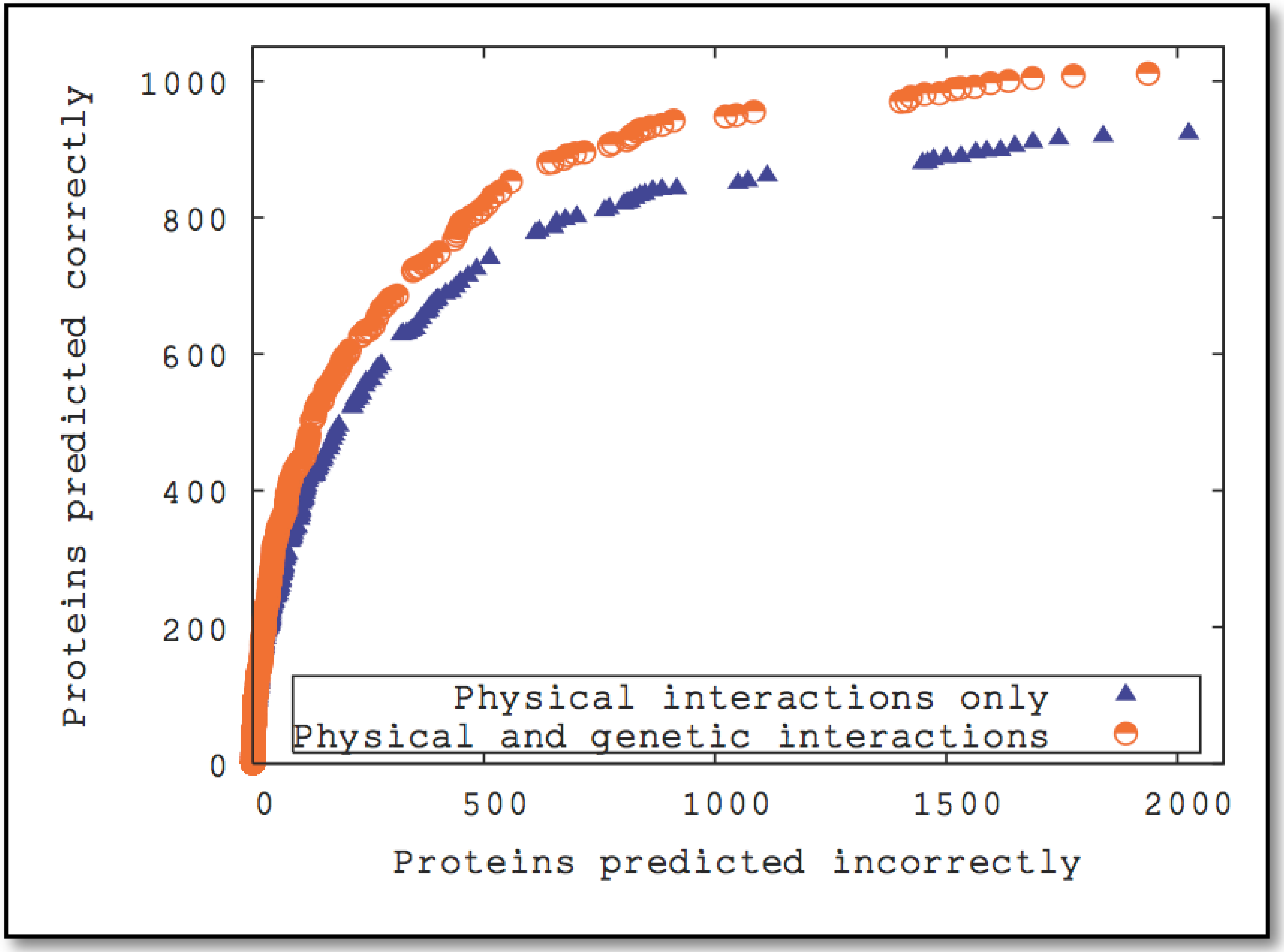
Additional Information



Use **genetic linkage** to modify the edge **weights**
→ better performance (also for Majority and GenMultiCut)

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Summary: **Static** PPI-Networks

"Proteins are **modular machines**" <=> How are they related to each other?

1) **Understand** "Networks"
   prototypes (ER, SF, …) and their properties (*P(k), C(k),* clustering, …)

2) Get the **data**
   experimental and theoretical techniques (Y2H, TAP, co-regulation, …),
   quality control and data integration (Bayes)

3) **Analyze** the data
   compare *P(k), C(k),* clusters, … to prototypes → highly modular, clustered
   with sparse sampling → PPI networks are not scale-free

4) **Predict** missing information
   network structure combined from multiple sources → functional annotation

Next step: environmental changes, cell cycle
→ **changes** (dynamics) in the PPI network – **how and why?**