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

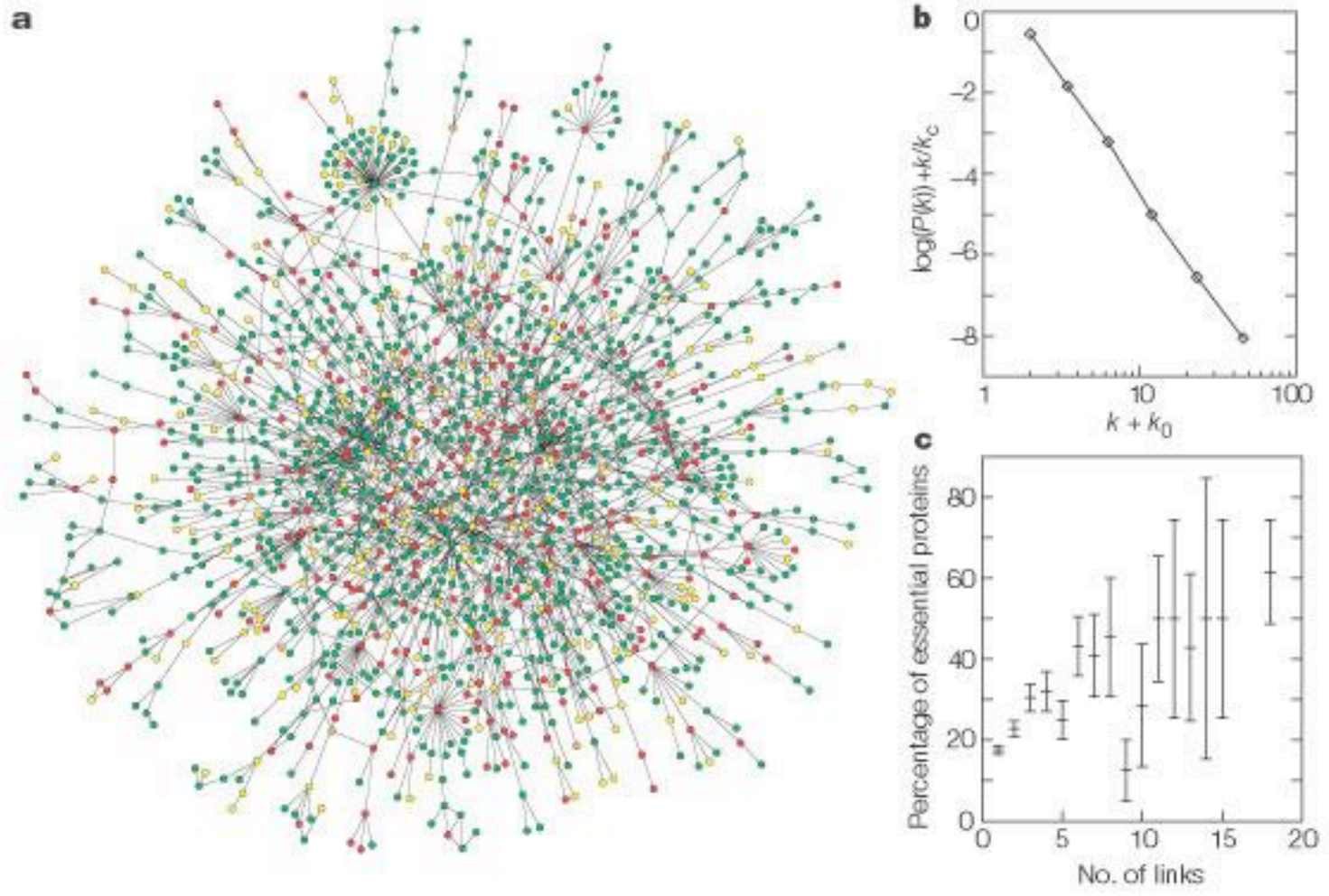
Mon, Nov 16, 2015

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
$\langle k \rangle$	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. $\langle k \rangle$ 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

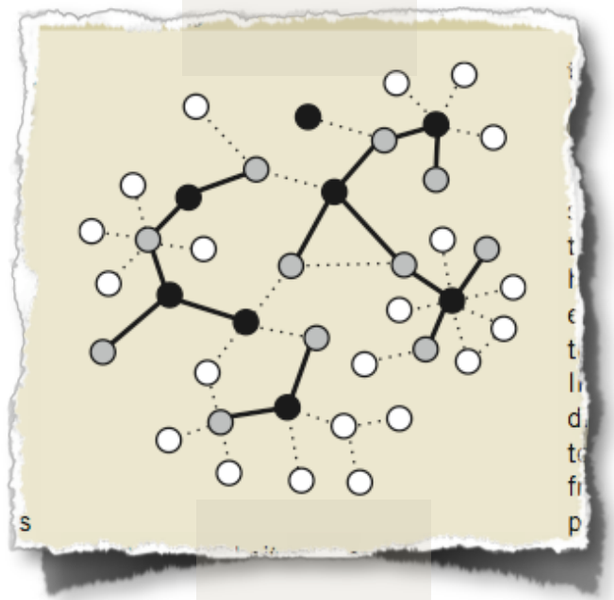
Effect of sampling on topology predictions of protein-protein interaction networks

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?

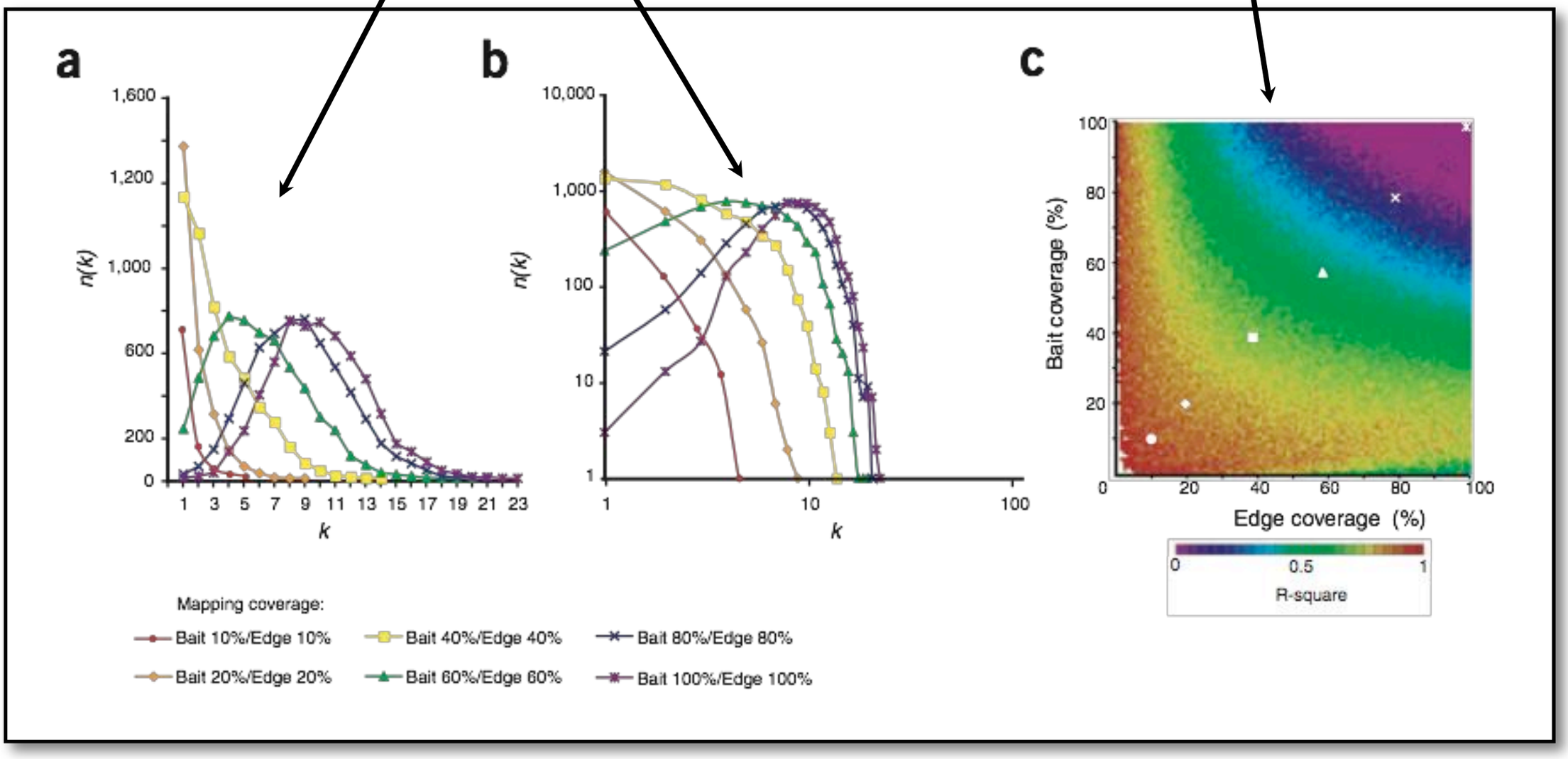
- Random (ER / Erdös-Renyi) → $P(k)$ = Poisson
- Exponential (EX) → $P(k) \sim \exp[-k]$
- scale-free / power-law (PL) → $P(k) \sim k^{-\gamma}$
- $P(k)$ = truncated normal distribution (TN)



Sparsely Sampled random (ER) Network

resulting *P(k)* for different coverages

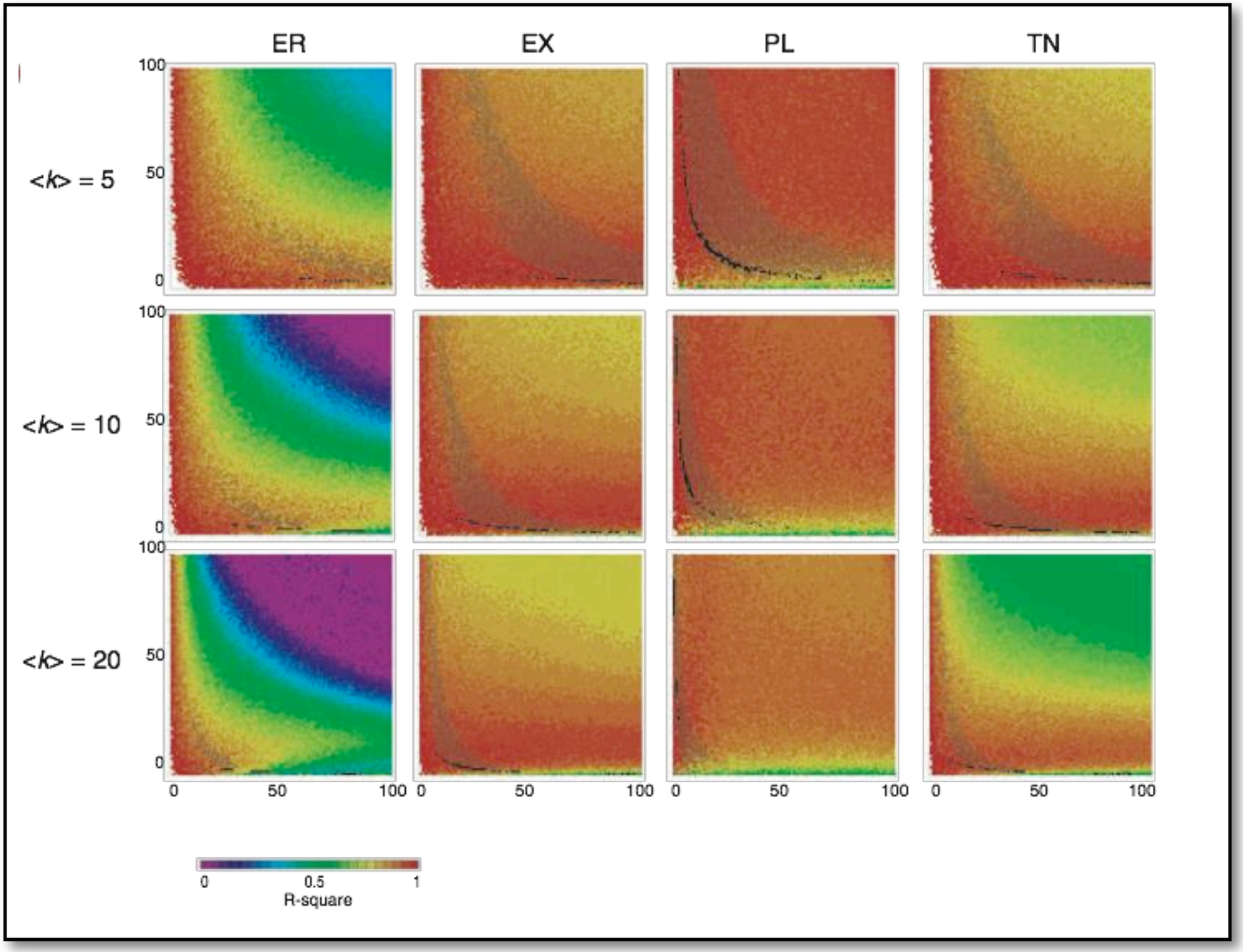
linearity between *P(k)* and power law



→ for **sparse** sampling (10-20%), even an ER networks
"looks" scale-free (when only *P(k)* is considered)

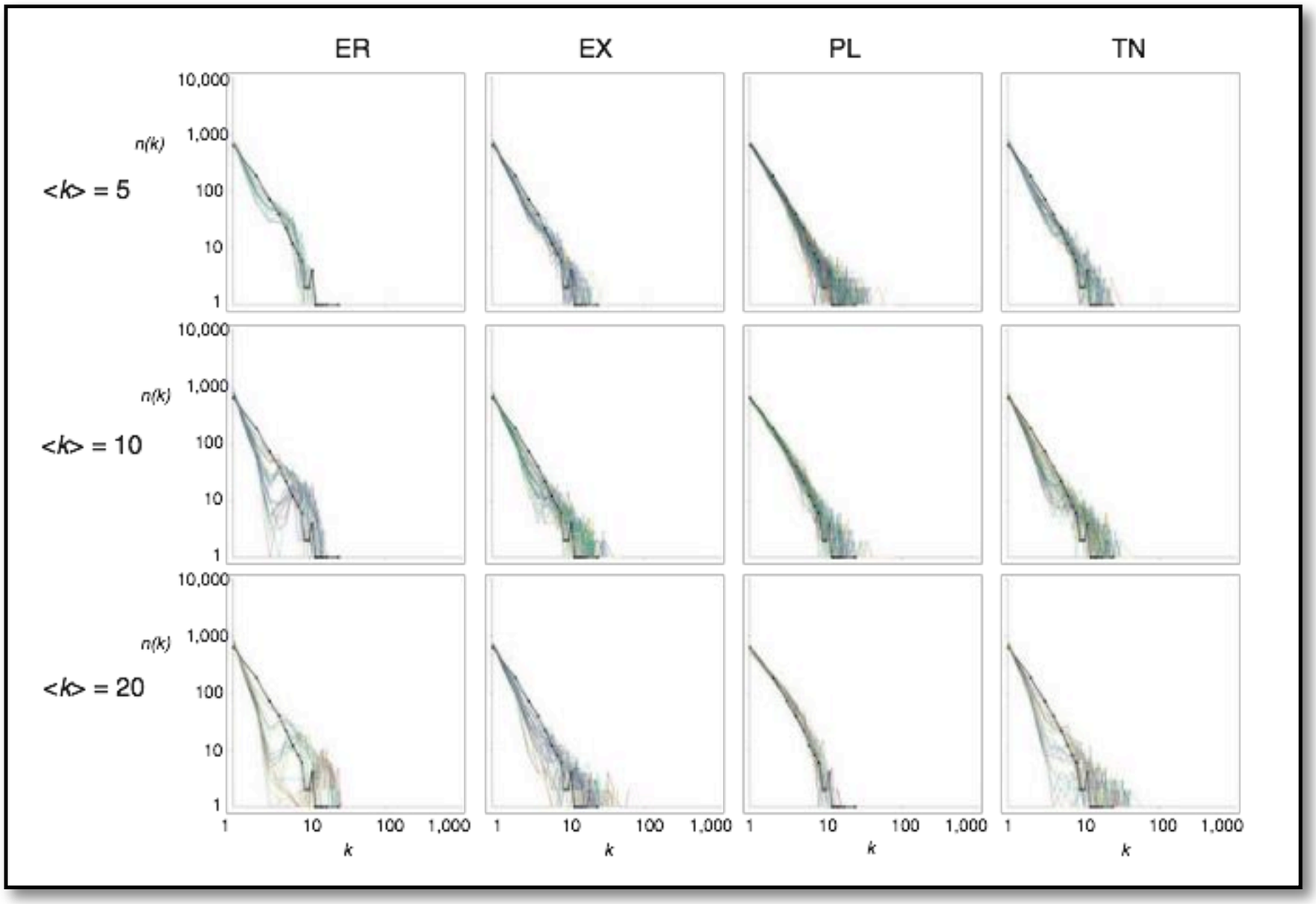
Han et al, *Nature Biotech* **23** (2005) 839

Anything Goes



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

Compare to Uetz et al. Data



Uetz et al. data (solid line) is compared to sampled networks of similar size.

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

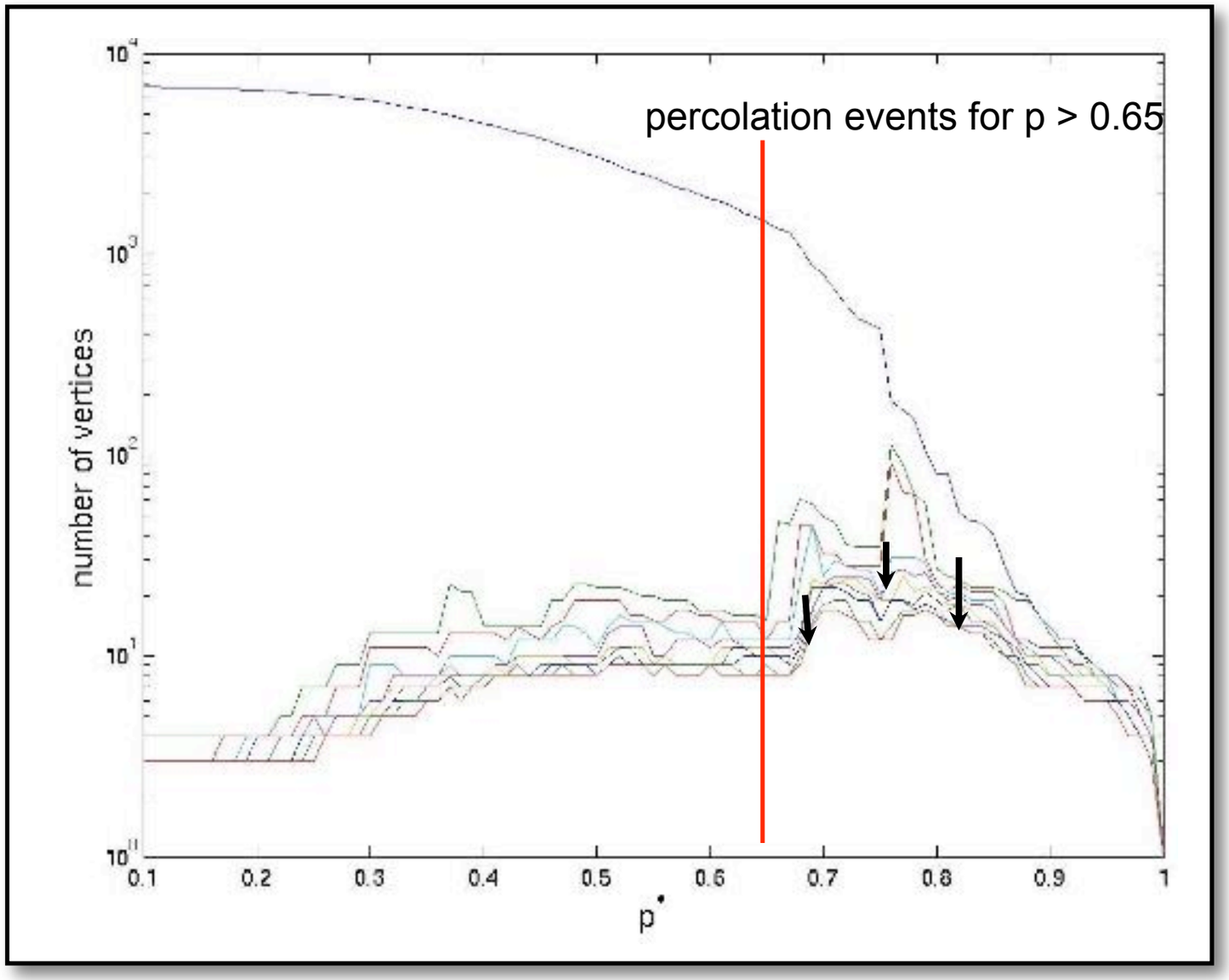
The Fly Network

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 nodes 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 (Albert-Barabasi)
AGV Aging vertices network
SMW Small world network

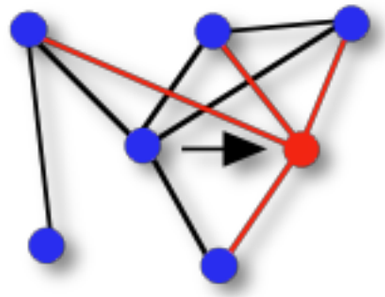
Growth Type 1: DMC

"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

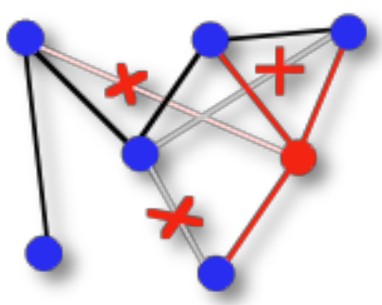
Algorithm:

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



- duplicate existing node with all interactions

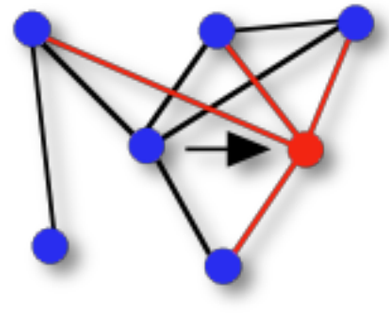
- for all neighbors: delete with probability q_{del} either link from original node **or** from copy



Growth Type 2: DMR

"Duplication with random mutations"

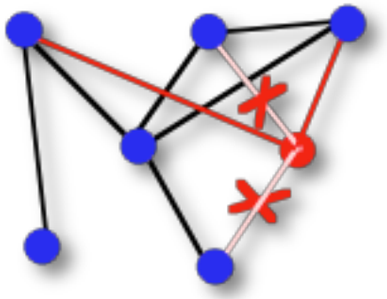
Gene duplication, but no correlation between original and copy (original unaffected by copy)

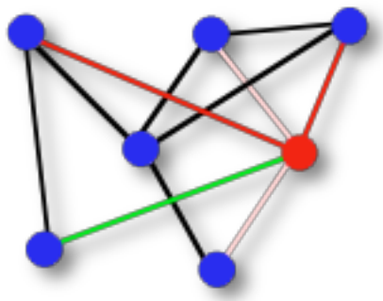


Algorithm:

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 $(k_i + \alpha)$, $\alpha = 0 \ldots 5$
(BA for $\alpha = 0$)

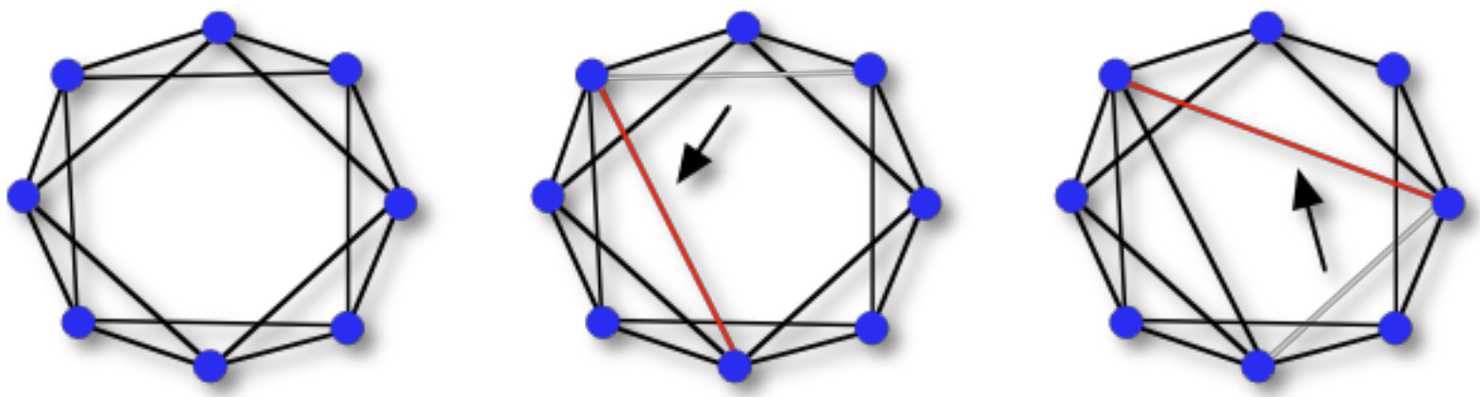
Growth Types 6-7: AGV and SMW

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

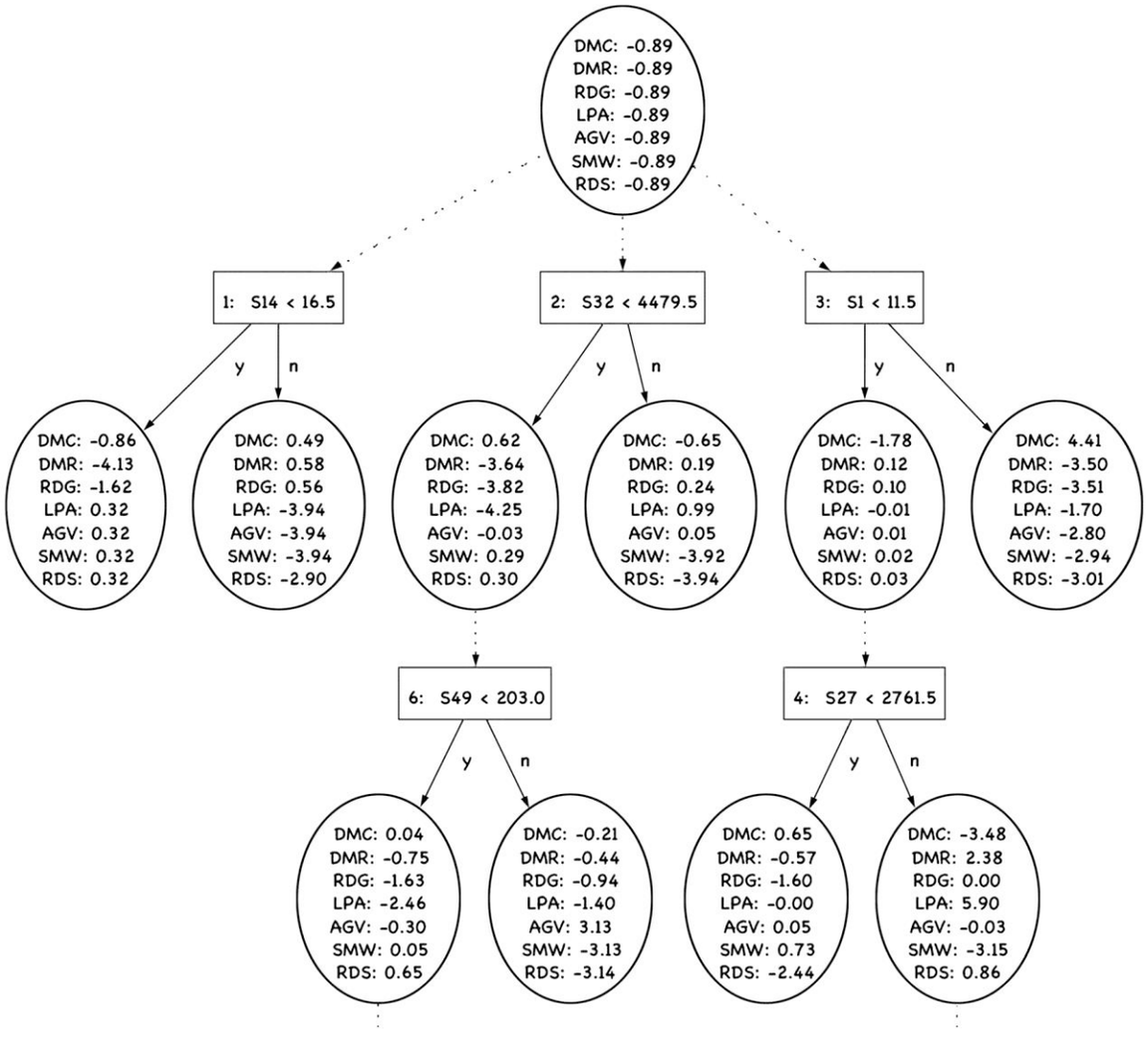
SMW = small world networks (Watts, Strogatz, *Nature* **363** (1998) 202)

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

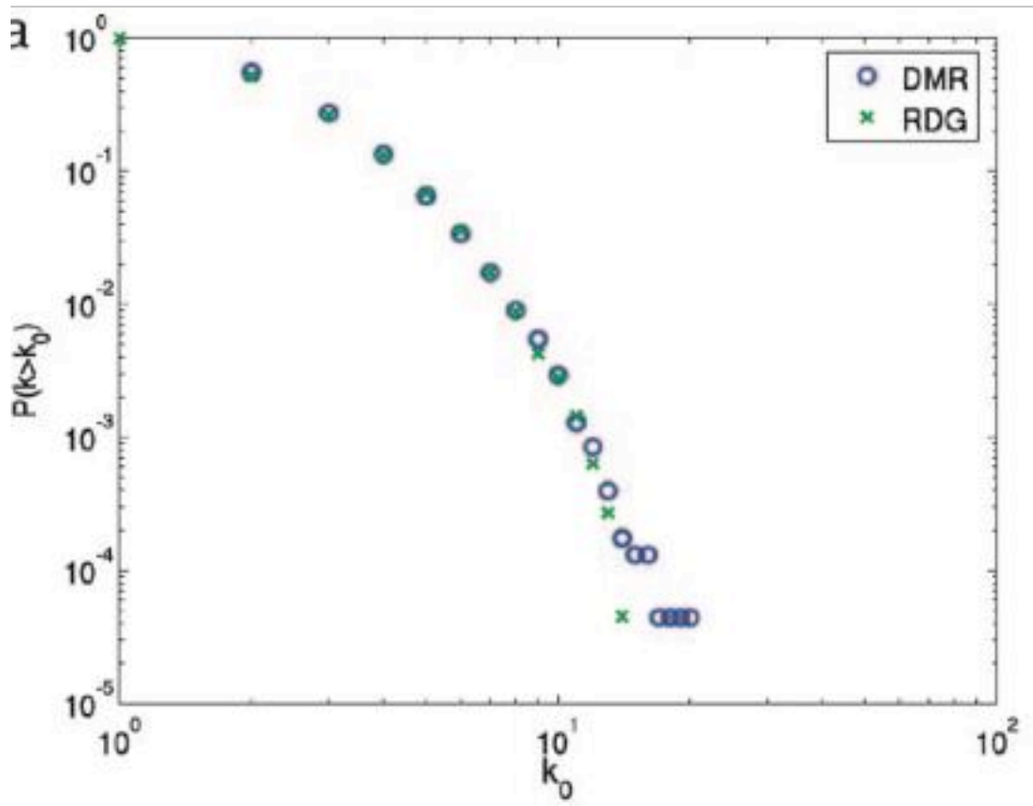
Decision nodes count occurrence of motifs

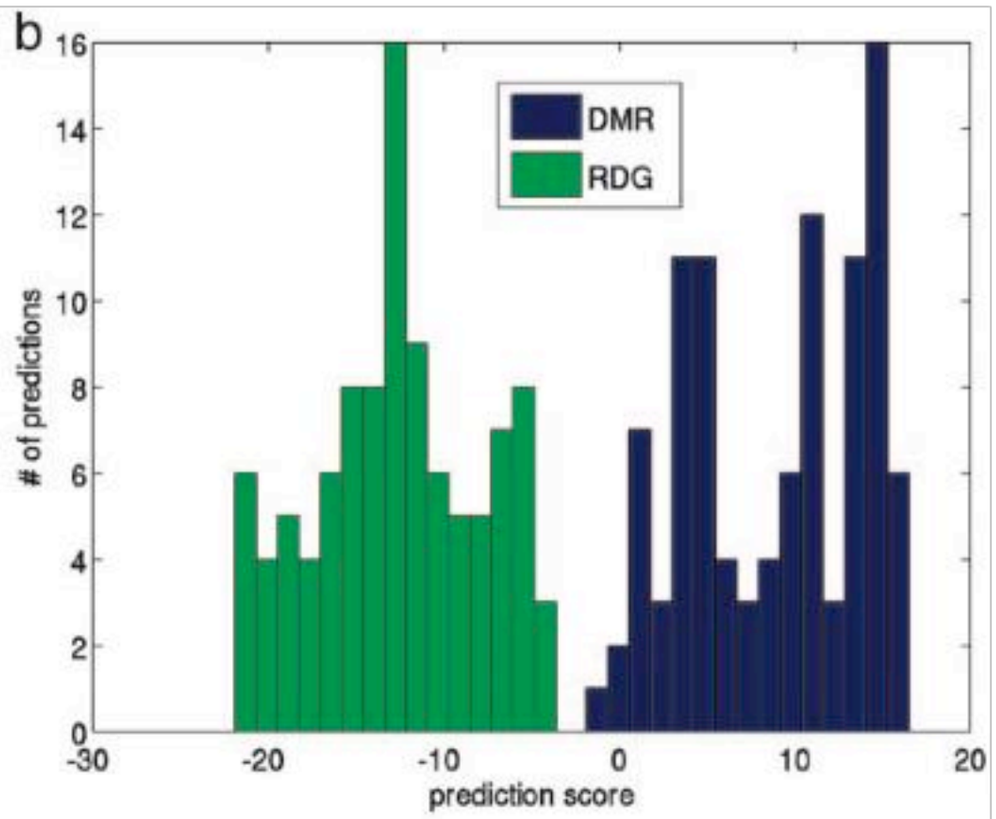
Prediction accuracy for networks similar to fly network with $p = 0.5$:

	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 <C> and <l> (left), but different counts of the network motifs (right)

-> networks can (only) 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

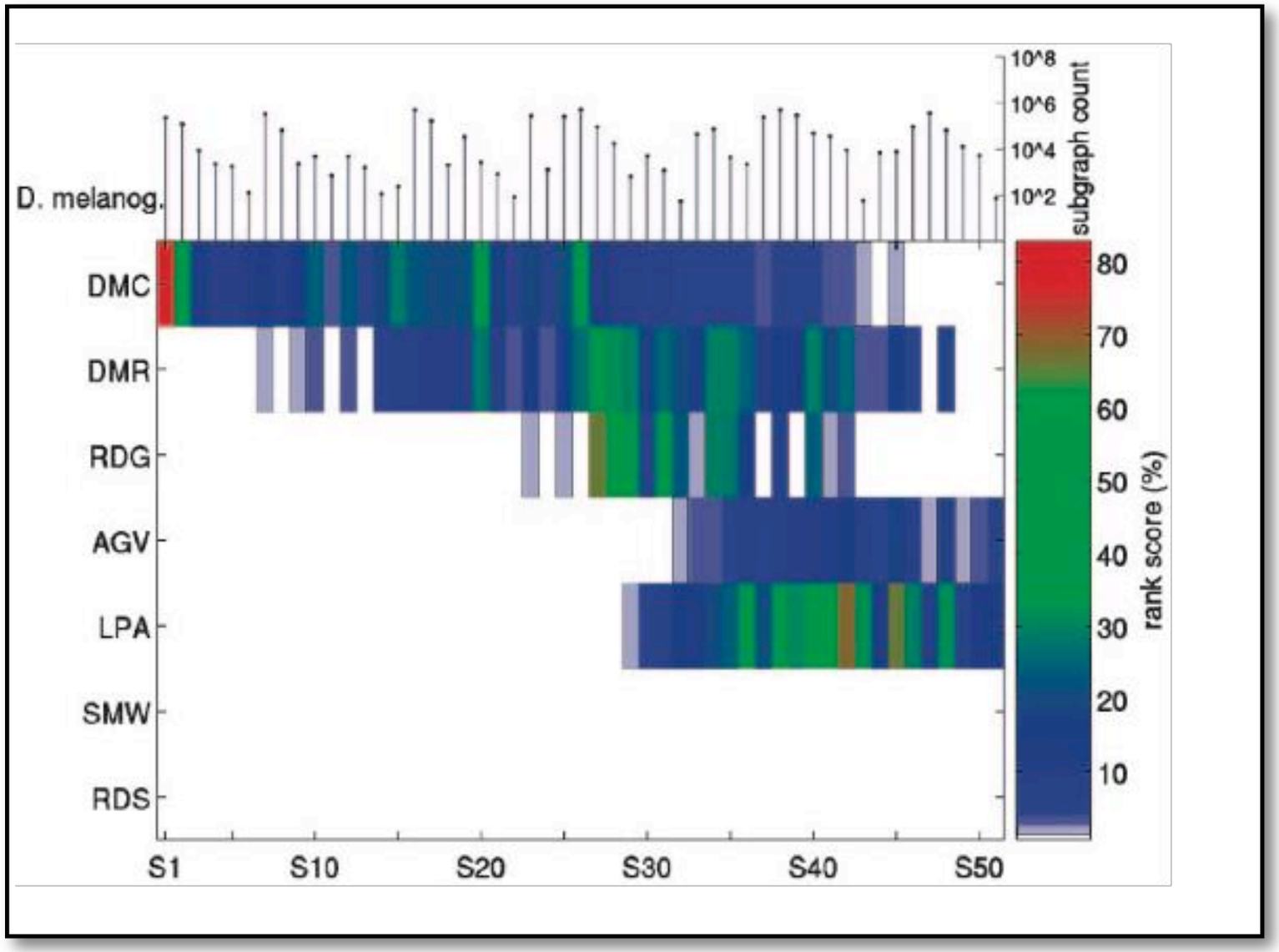
Drosophila is consistently (independently of the cut-off in subgraph size) classified as a DMC network, with an especially strong prediction for a confidence threshold of $p^* = 0.65$.

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

→ Scale-free or random networks are very unlikely

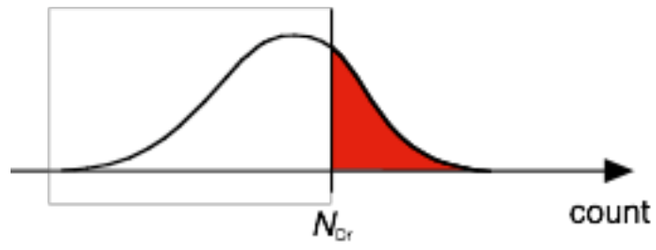
Middendorf et al, *PNAS* **102** (2005) 3192

Motif Count Frequencies



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

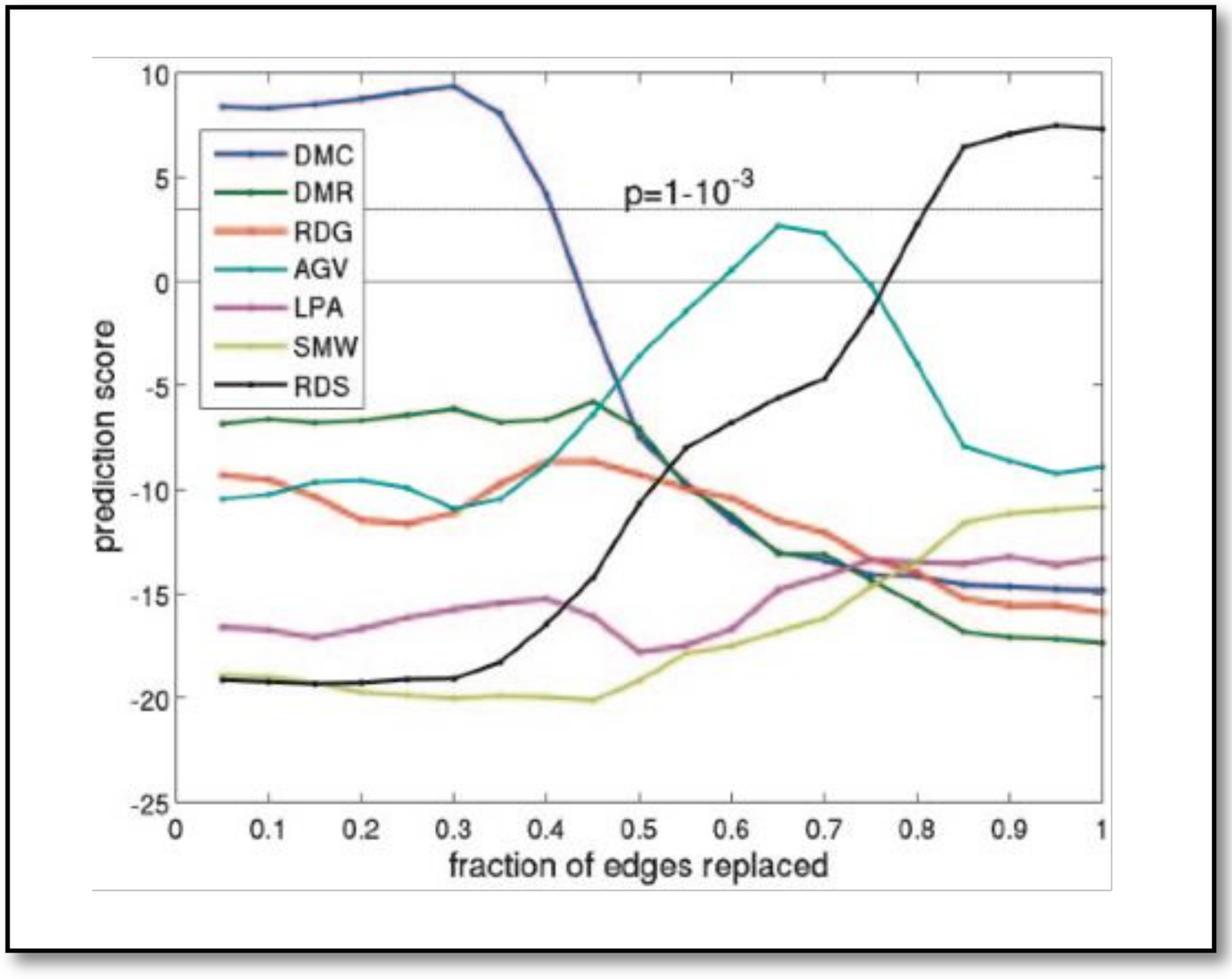
rank score: fraction of test networks with a higher count than Drosophila (50% = same count as fly on avg.)



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

Experimental Errors?

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



→ Classification **unchanged** for **≤ 30%** incorrect edges,
at higher values RDS takes over (as to be expected)

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?

BRENDA
The Comprehensive Enzyme Information System

TU Braunschweig
Dept. of Bioinformatics

EC **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?

BIOINFORMATICS

Vol. 21 Suppl. 1 2005, pages i302–i310
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,]*

[1]Computer Science Department and [2]Lewis-Sigler Institute for Integrative Genomics, Princeton University, Princeton, NJ 08544, USA

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.

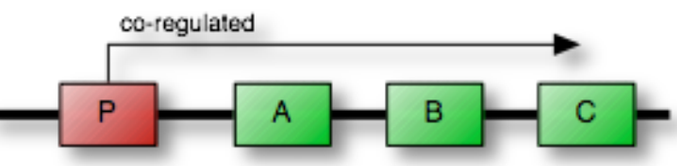
2946 have a MIPS functional annotation

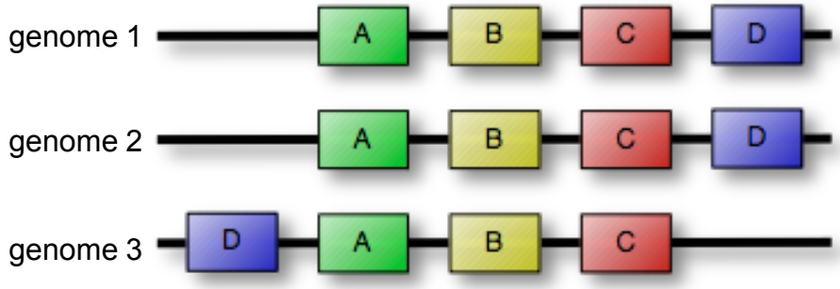
Partition the Graph

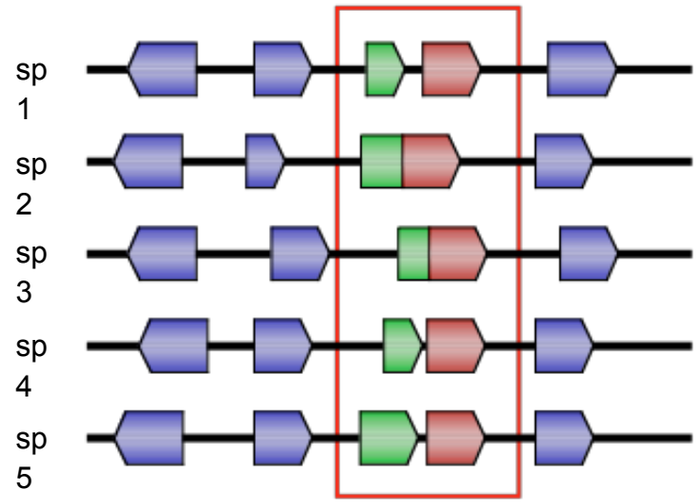
Large **PPI networks** were built from:

- 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 | help / support | contribute | downloads | mirrors | about us

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.

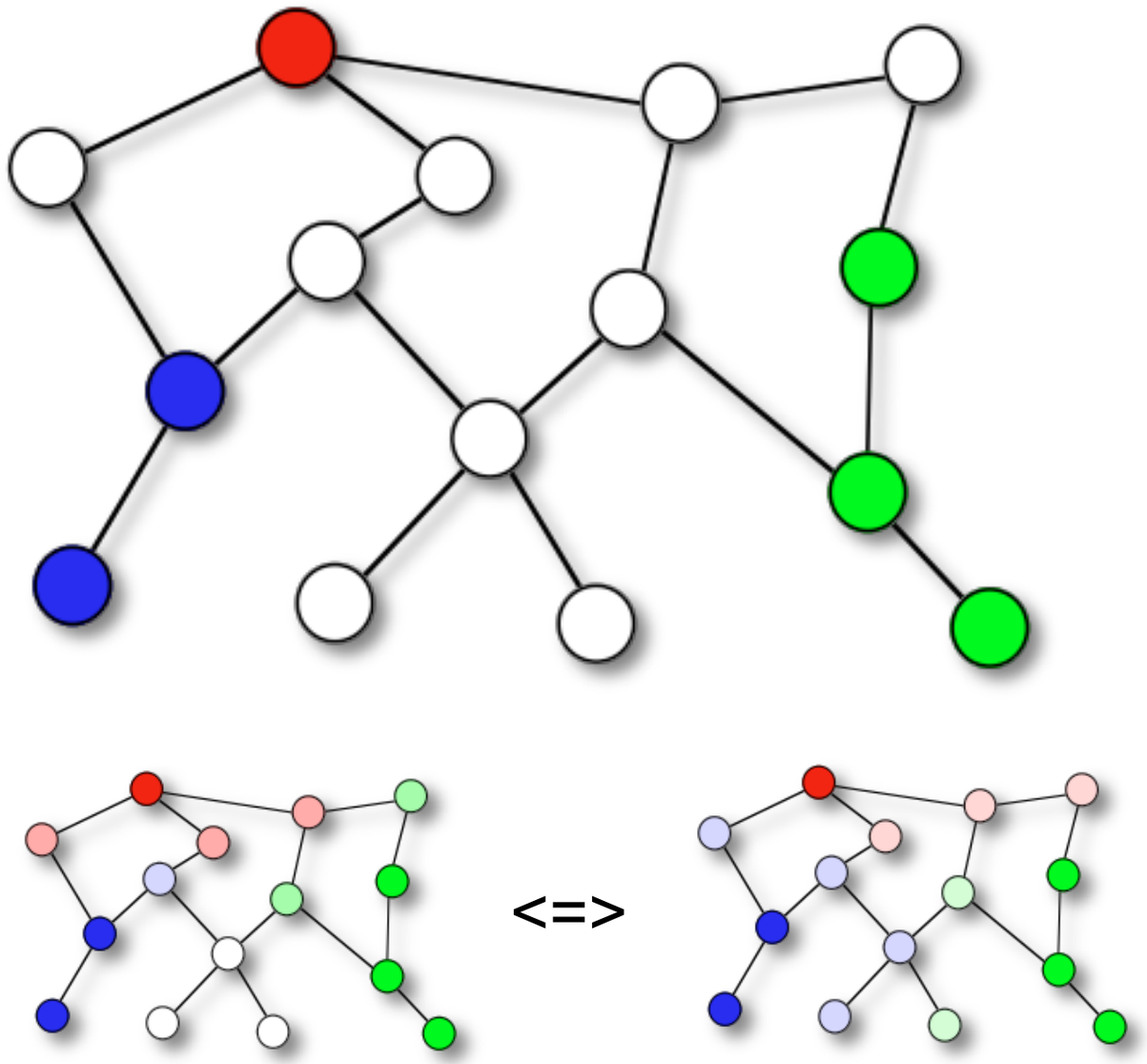
BioGRID Links

- 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



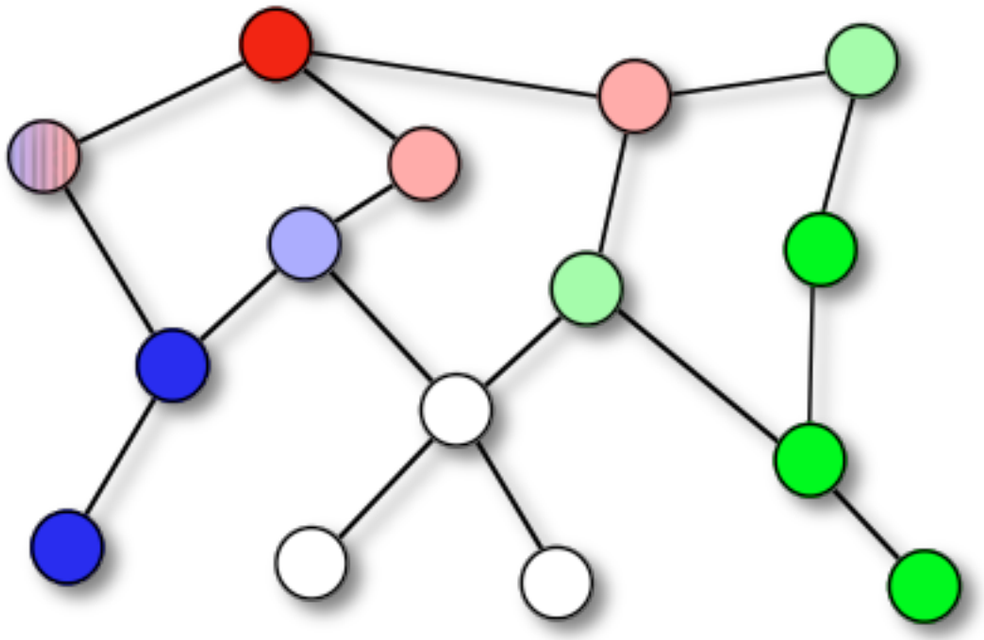
Similar:
How to **assign colors** to the white nodes?

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



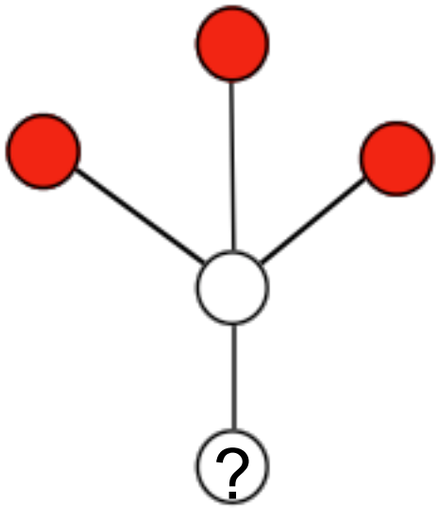
Majority makes only limited use of the local connectivity
→ cannot assign function to next-neighbors

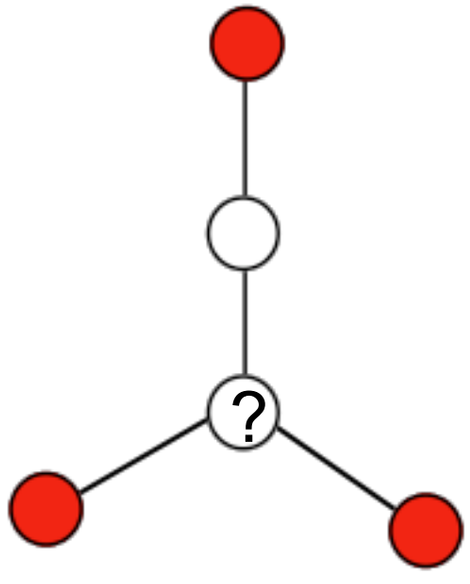
For weighted graphs:
→ weighted sum

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 algorithm does not consider local network topology

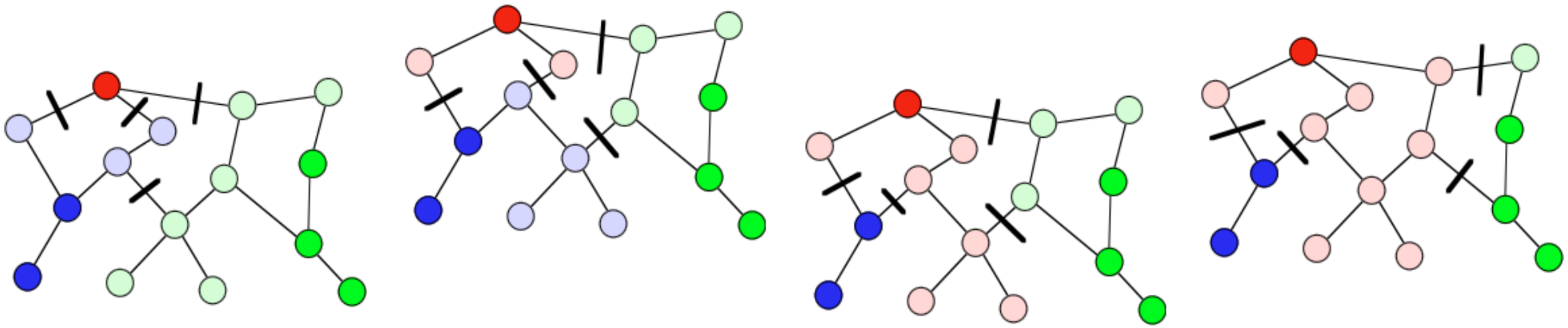
Both examples (left) are treated **identically** with $r = 2$

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 $g_0^a(u,v) = 0$

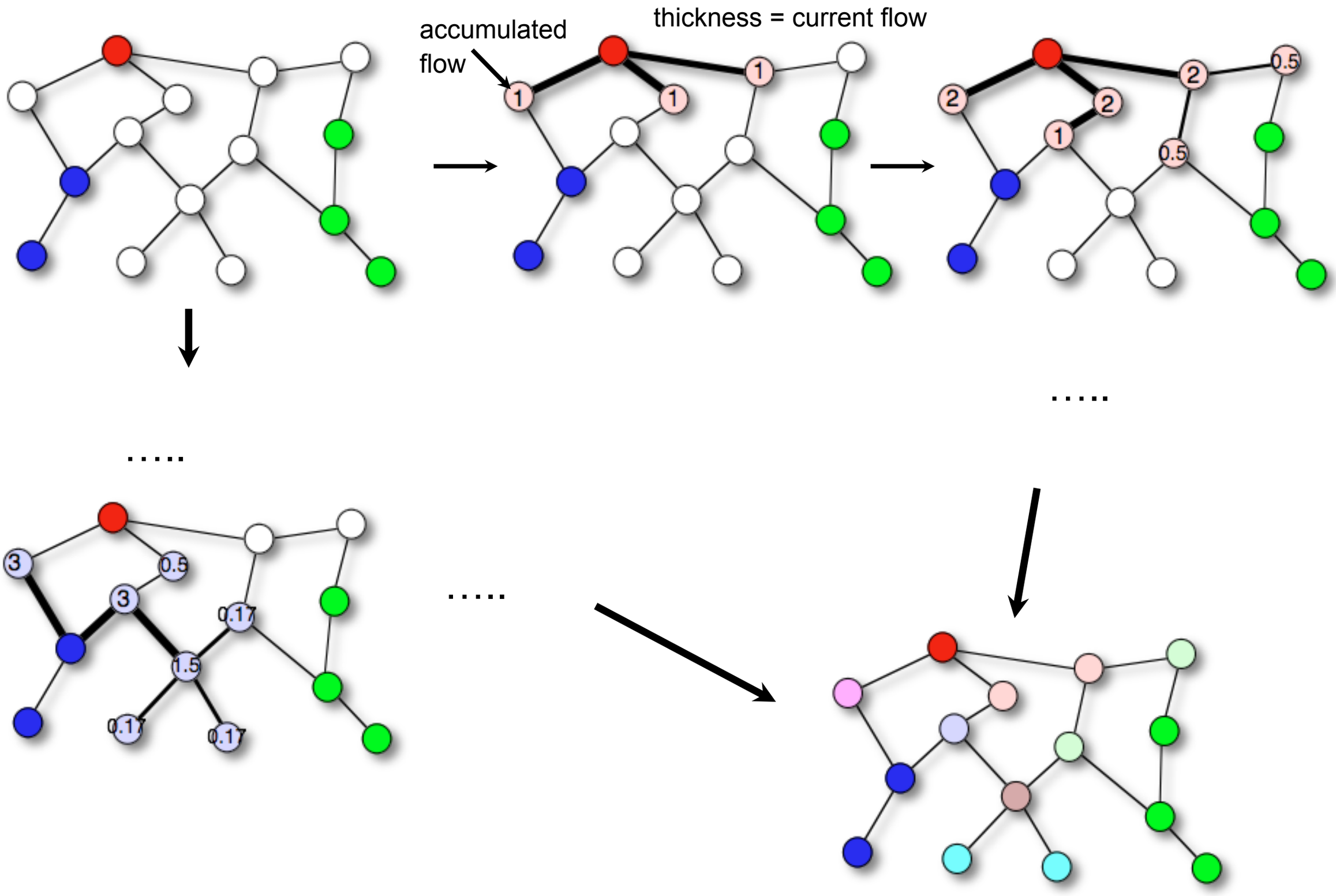
Then: **downhill flow** with capacity constraints

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

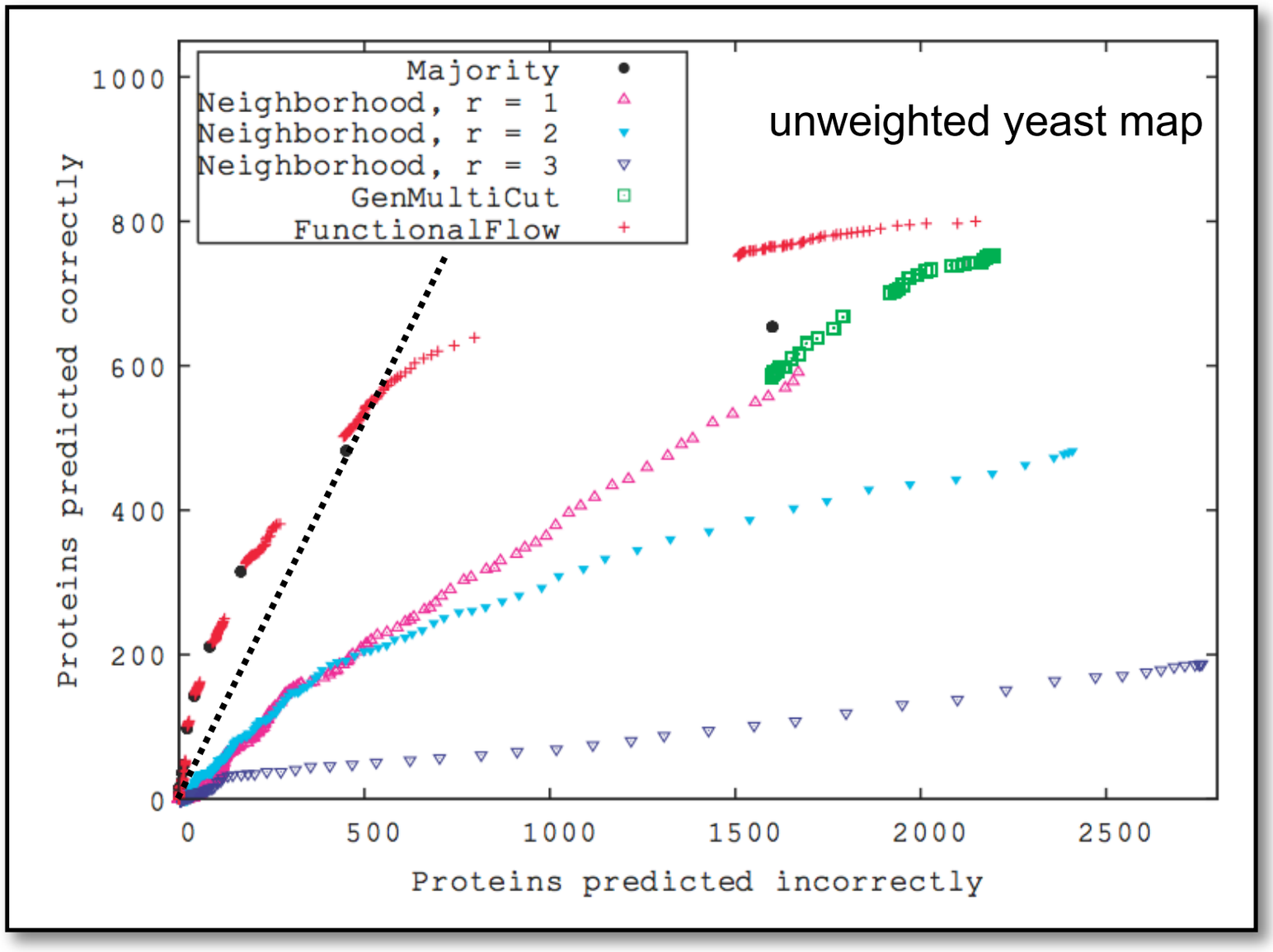
Score from accumulated in-flow: $f_a(u) = \sum_{t=1}^{d} \sum_{v:(u,v)\in E} g_t^a(v,u)$

Nabieva et al, Bioinformatics 21 (2005) i302

An Example


accumulated
flow
thickness = current flow
1
1
1
2
2
2
1
0.5
0.5
.....
.....
3
0.5
3
0.17
1.5
0.17
0.17

Comparison



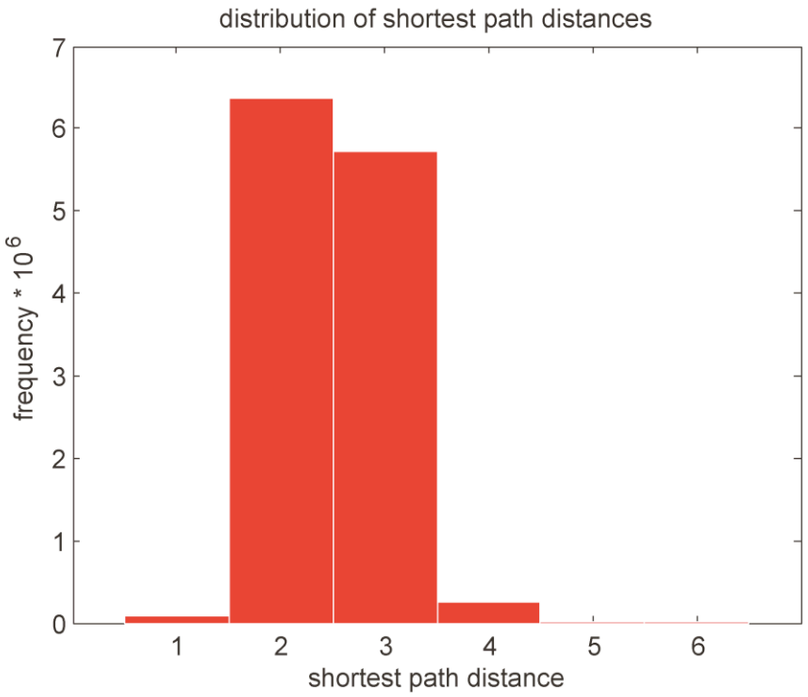
For FunctionalFlow:
six propagation steps
(diameter of the yeast
network ≈ 12)

Change **score threshold** for accepting annotations → ratio **TP/FP**
→ **FunctionalFlow** performs **best** in the high-confidence region
→ but many false predictions!!!

Nabieva et al, Bioinformatics 21 (2005) i302

Going the Distance for Protein Function Prediction: A New Distance Metric for Protein Interaction Networks

Citation: Cao M, Zhang H, Park J, Daniels NM, Crovella ME, et al. (2013) Going the Distance for Protein Function Prediction: A New Distance Metric for Protein Interaction Networks. PLoS ONE 8(10): e76339. doi:10.1371/journal.pone.0076339



(a)

Relying on the ordinary shortest-path distance metric in PPI networks is problematic because PPI networks are “small world” networks.
Most nodes are close to all other nodes.

→ any method that infers similarity based on proximity will find that a large fraction of the network is proximate to any typical node.

(b)

Largest connected component of *S. cerevisiae* PPI network (BioGRID) has 4990 nodes and 74,310 edges (physical interactions).
Fig. shows the histogram of shortest-path lengths from this network. Over 95% of all pairs of nodes are either 2 hops or 3 hops apart

What nodes mediate short contacts?

The 2-hop neighborhood of a typical node probably includes around half of **all nodes** in the graph.

One of the **reasons** that paths are typically short in biological networks like the PPI network is due to the **presence of hubs**.

Hubs often represent proteins with *different* functional roles than their neighbors.

Hubs are also more likely to be proteins with multiple, distinct functions.

$\rightarrow$ not all short paths provide equally strong evidence of similar function in PPI networks.

DSD Distance Metric

Given some fixed $k>0$, we define $He^{\{k\}}(A,B)$ to be the expected number of times that a random walk starting at A and proceeding for k steps, will visit B.

Consider the undirected graph $G(V,E)$ on the vertex set $V=\{v_1,v_2,v_3,\ldots,v_n\}$ and $|V|=n$.

$$He(v_i)=(He(v_i,v_1),He(v_i,v_2),\ldots,He(v_i,v_n))$$

$$DSD(u,v)=||He(u)-He(v)||_1$$

$||He(u)-He(v)||_1$ denotes the L_1 norm of the He vectors

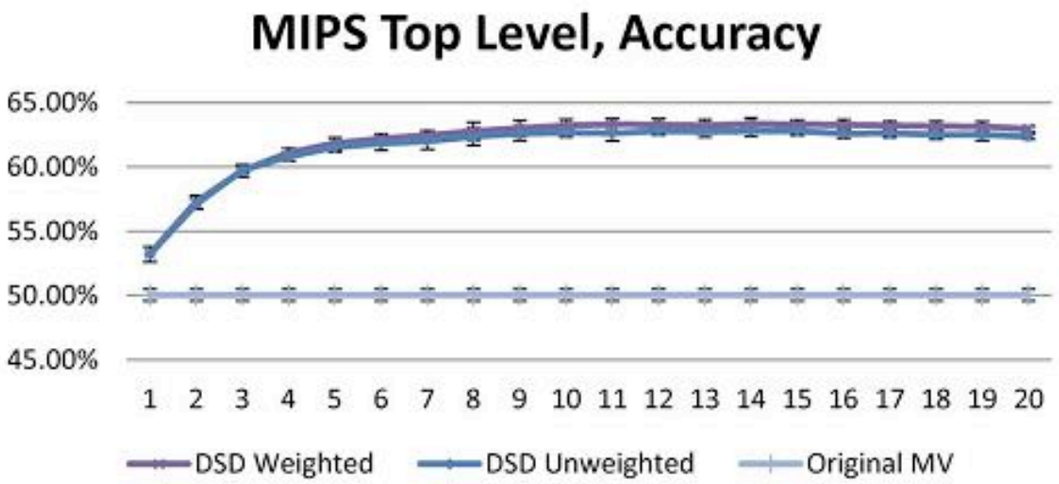
The one-norm (also known as the L_1-norm, ℓ_1 norm, or mean norm) of a vector $\vec{v}$ is denoted $\|\vec{v}\|_1$ and is defined as the sum of the absolute values of its components:

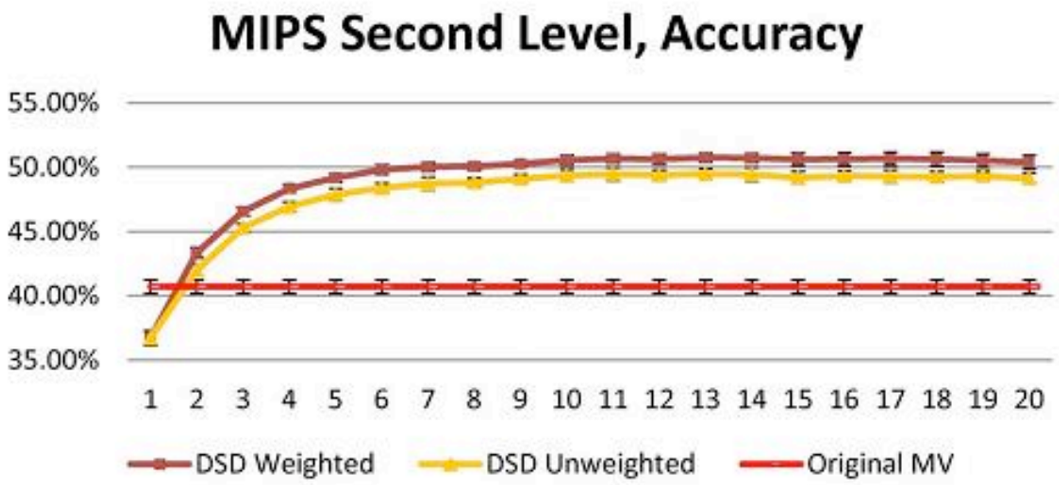
$$\|\vec{v}\|_1 = \sum_{i=1}^{n} |v_i| \tag{1}$$

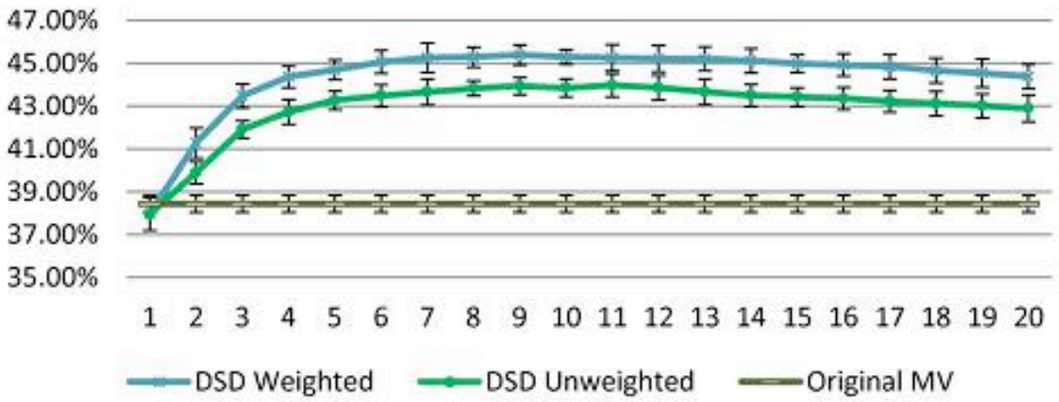
for example, given the vector $\vec{v} = (1, -4, 5)$, we calculate the one-norm:

$$\|(1,-4,5)\|_1 = |1| + |-4| + |5| = 10$$

DSD clearly improves functional predictions







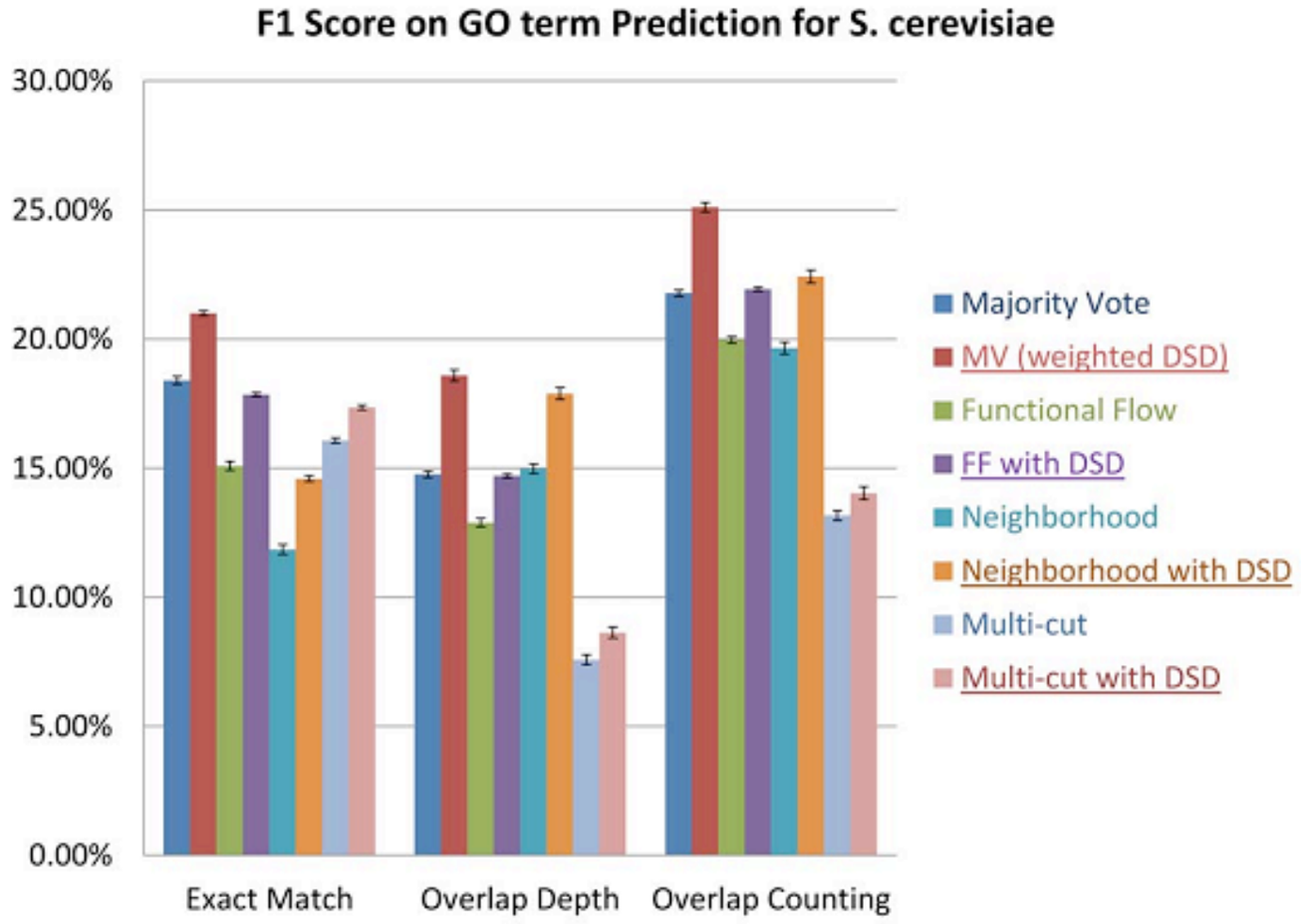


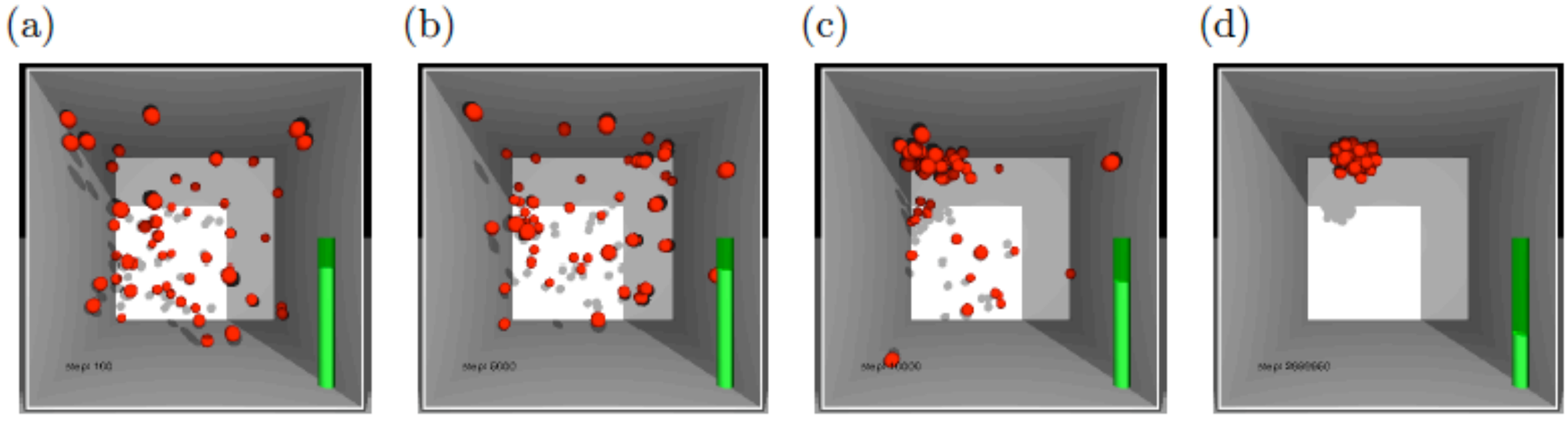
Figure 6. Improvement on F1 Score for DSD using three evaluation methods: exact match, overlap depth and overlap counting, on informative GO terms for the four algorithms for *S. cerevisiae* in 10 runs of 2-fold cross validation.

MV: majority voting

What you can else do with Interaction graphs?

E.g. efficiently tracking interactions between many particles In dynamics simulations

Strongly attracting particles form large “blob”



How can one analyze the particle connectivity efficiently?

```
For i = 1 to N-1
        For j = i to N
                For k = j to N
                        If (i .is bound to. j) then
                                If (j .is bound to. k) then ….
```

this is impractical!

M.Sc. thesis Florian Lauck (2006)

Map simulation to interaction graph

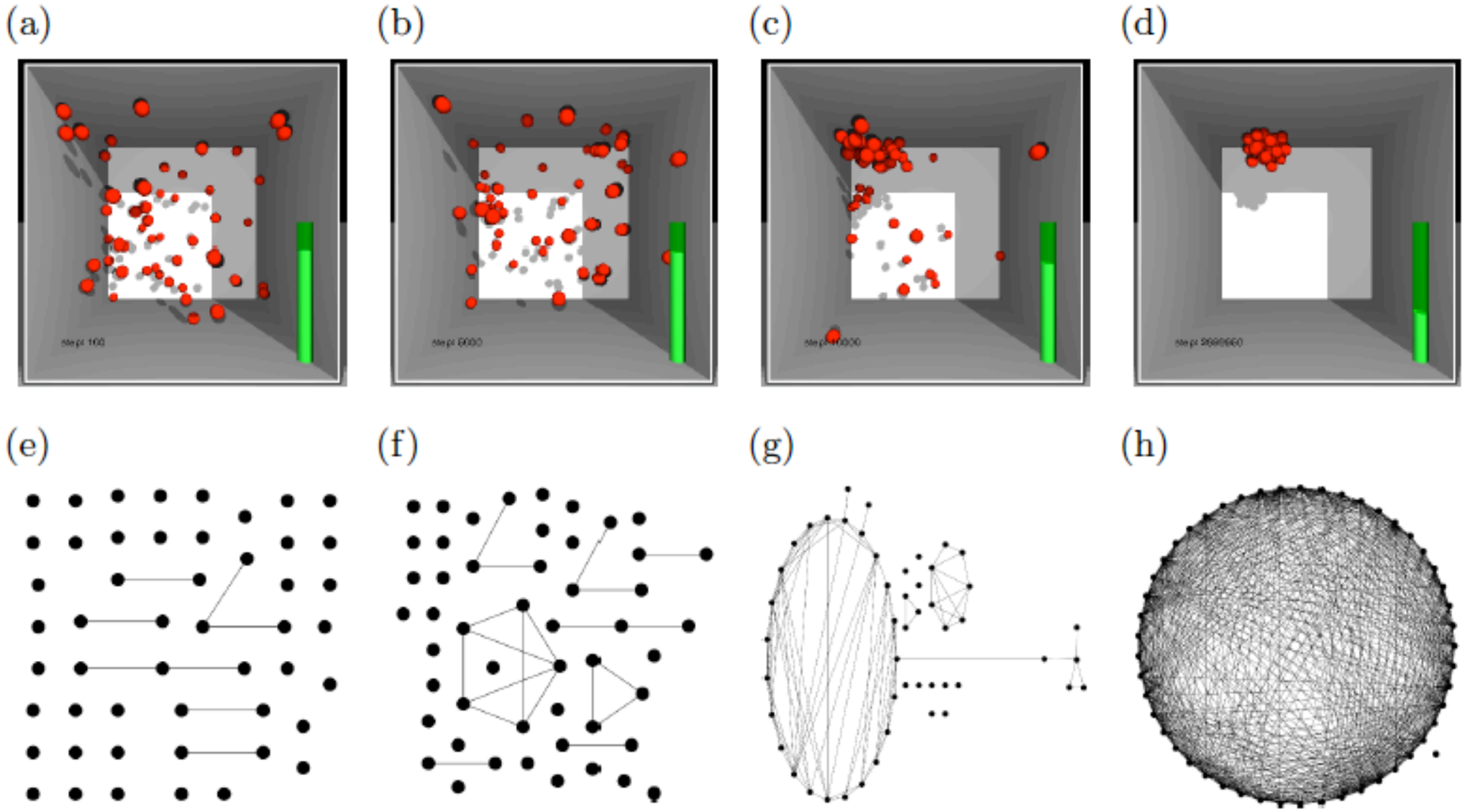
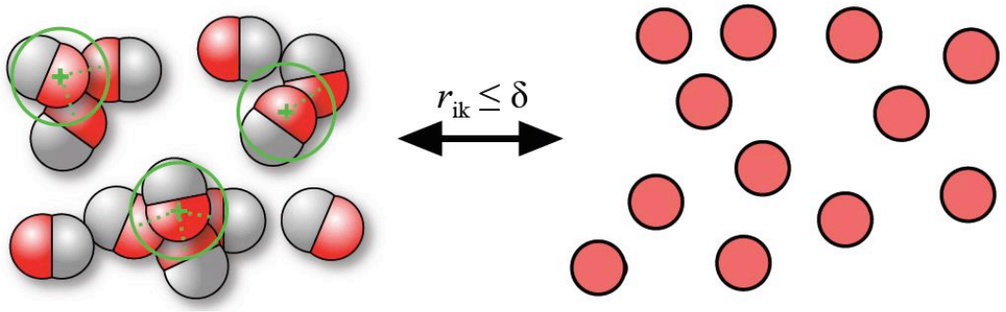


Figure 2.7: Graph and spatial view of a simulation with 50 particles at four different points in time. The green bar denotes the energy of the system.

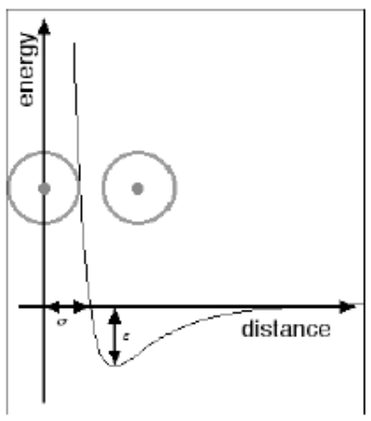
M.Sc. thesis Florian Lauck (2006)

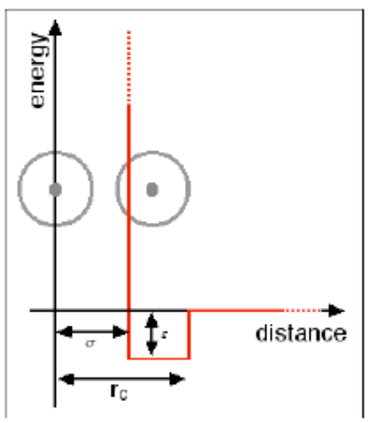
Large number of simultaneous assocications: map simulations to interaction graphs

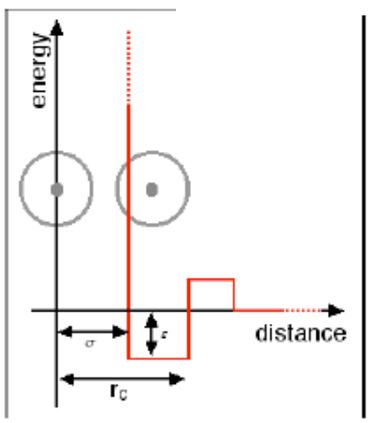


Simple MC scheme for diffusion + association/ dissociation

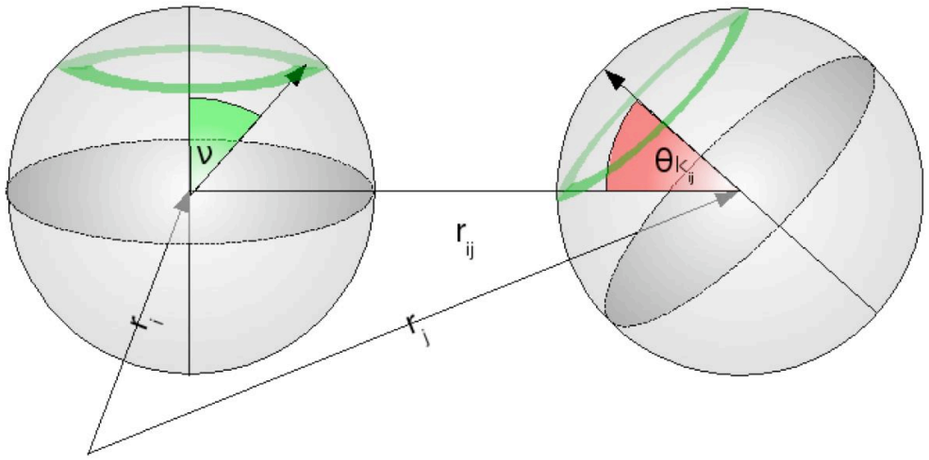
```
function INITIALIZE(N)
    for P ∈ List of Particles do
        CREATE RANDOM COORDINATES(P)
    CREATE GRAPH(N)
for all Iterations do
    for P ∈ List of Particles do
        MOVE AND ROTATE(P)
        for all  P_i ∈ (List of Particles - P) do
            d = DISTANCE(P, P_i)
            e_i = POTENTIAL(d)
            if d ≤ r_C then APPEND(List of Interactions, (P, P_i))
            E_new += e_i
        a = TRANSITION PROBABILITY(E_new, E_old)
        x = RANDOMNUMBER
        if x ≤ p then                                   ▷ accept new state
            APPEND(List of ALL interactions, List of Interactions)
            E_old = E_new
        else                                            ▷ discard new state
            RESET(P) CLEAR(List of Interactions)
    UPDATE(Graph, List of ALL Interactions)
ANALYSIS(Graph)
```







Interaction patches define complex geometry

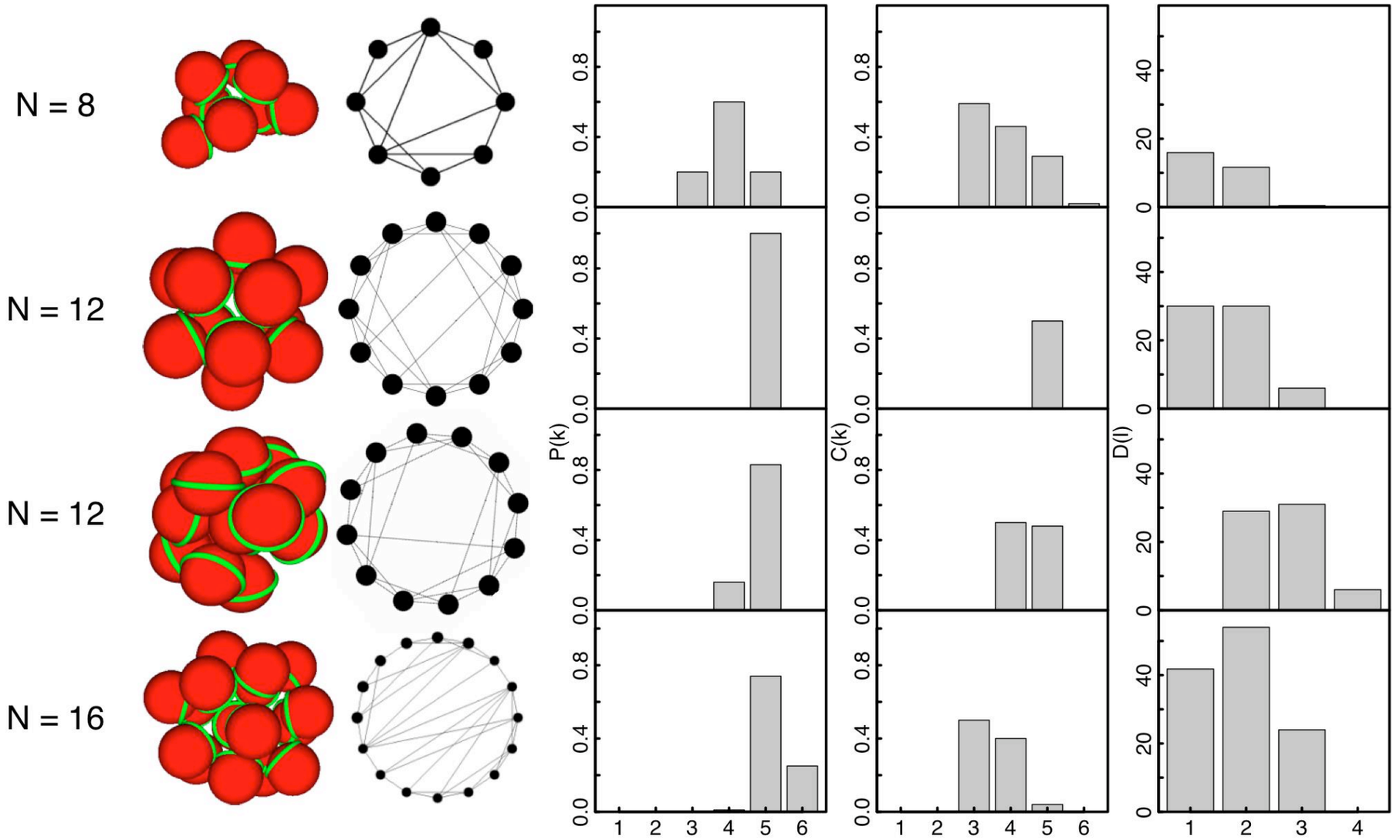


$$G_{ij}(r_{ij}, \theta_{ij}) = \exp\left[\frac{(\theta_{ij} - \nu)}{2\sigma^2_{PW}}\right]$$

$$V_{total} = V(r_{ij}) \times G_{ij}(r_{ij}, \theta_{ij}) \times G_{ji}(r_{ij}, \theta_{ji})$$

Lauck et al. , *JCTC 5, 641* (2009)

Assembly of icosahedral complexes



$$P(k) = \frac{N(k)}{N} \quad C(k) = \frac{\sum C_k}{n_k}$$

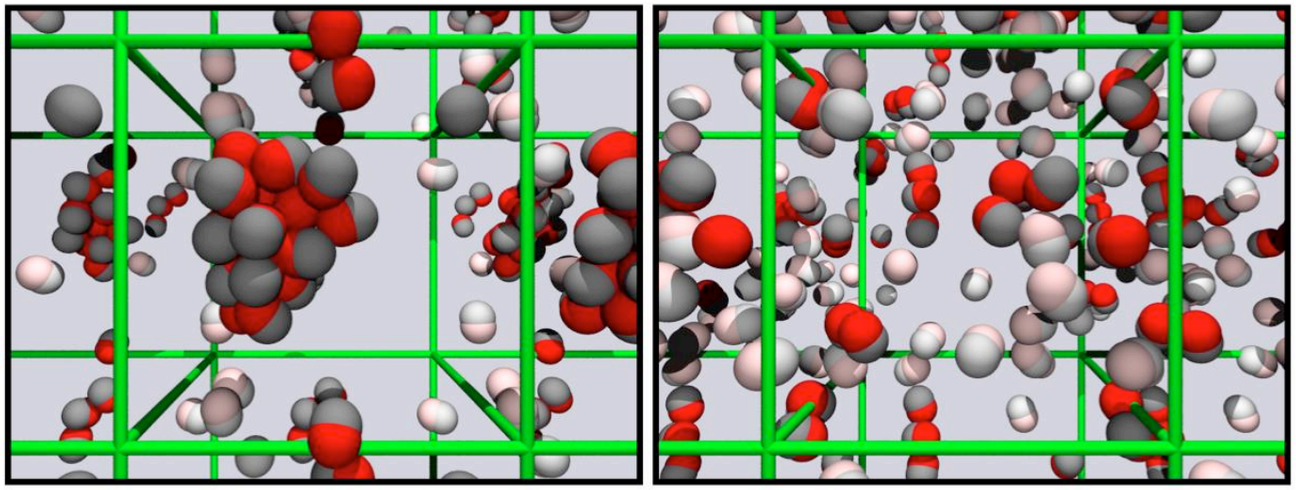
Degree distribution

Average Cluster coefficient

shortest pathways between nodes

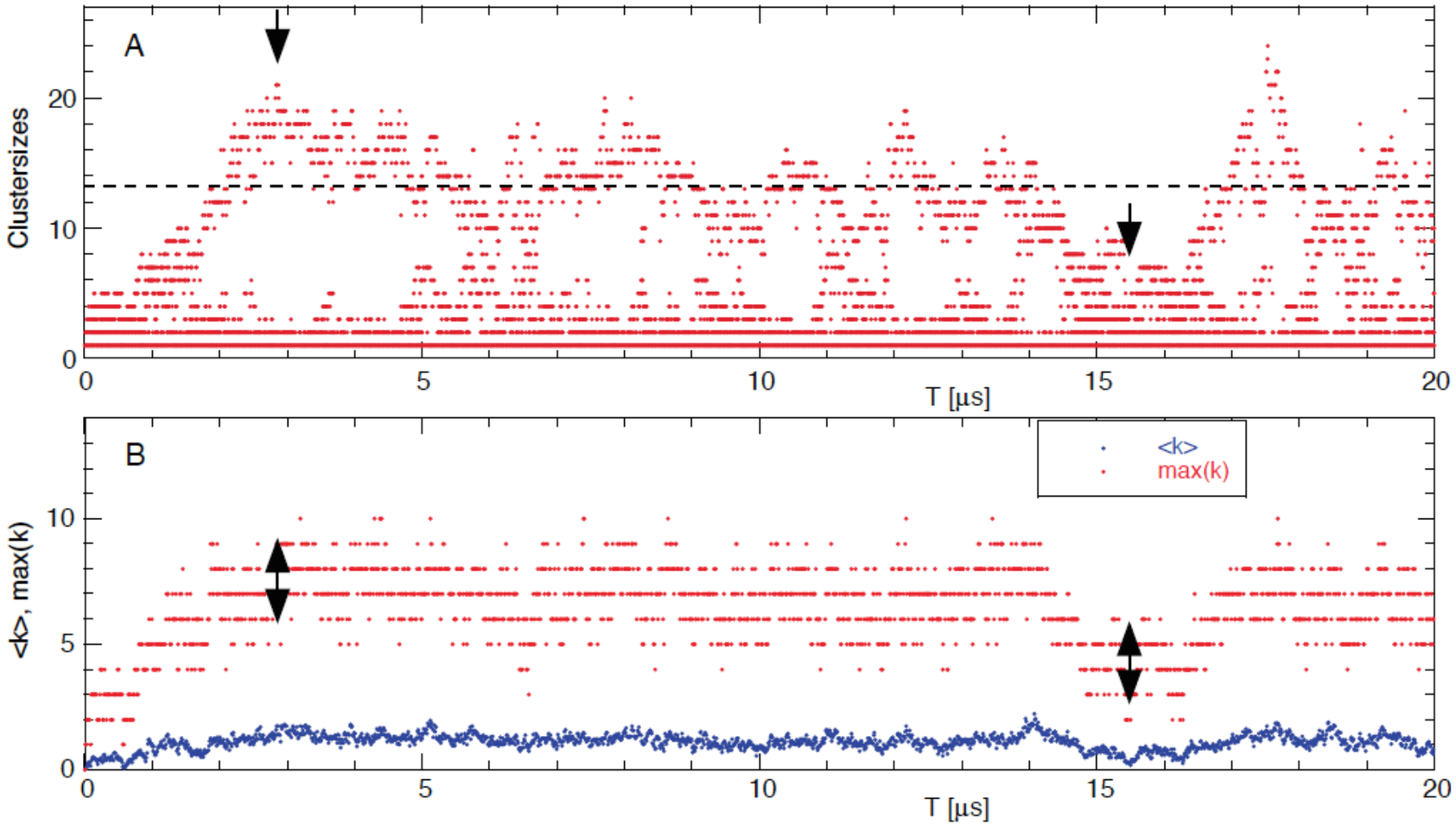
Lauck et al. , *JCTC 5, 641* (2009)

Dynamical view at particle agglomeration



T = 2.85 µs

T = 15.44 µs



Two snapshots

T = 2.85 µs
most of the
particles are part
of a large cluster,

T = 15.44 µs
largest cluster
has 3 particles.

Geyer,

BMC Biophysics (2011)

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?**