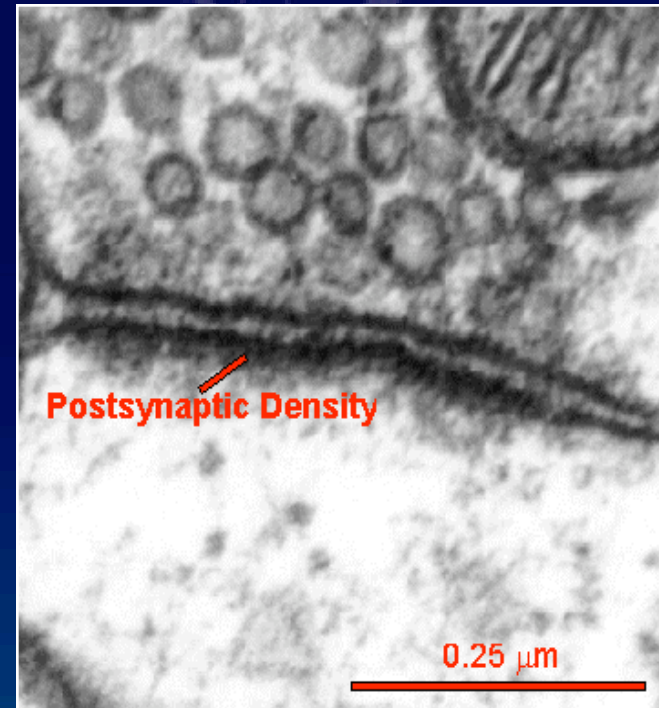
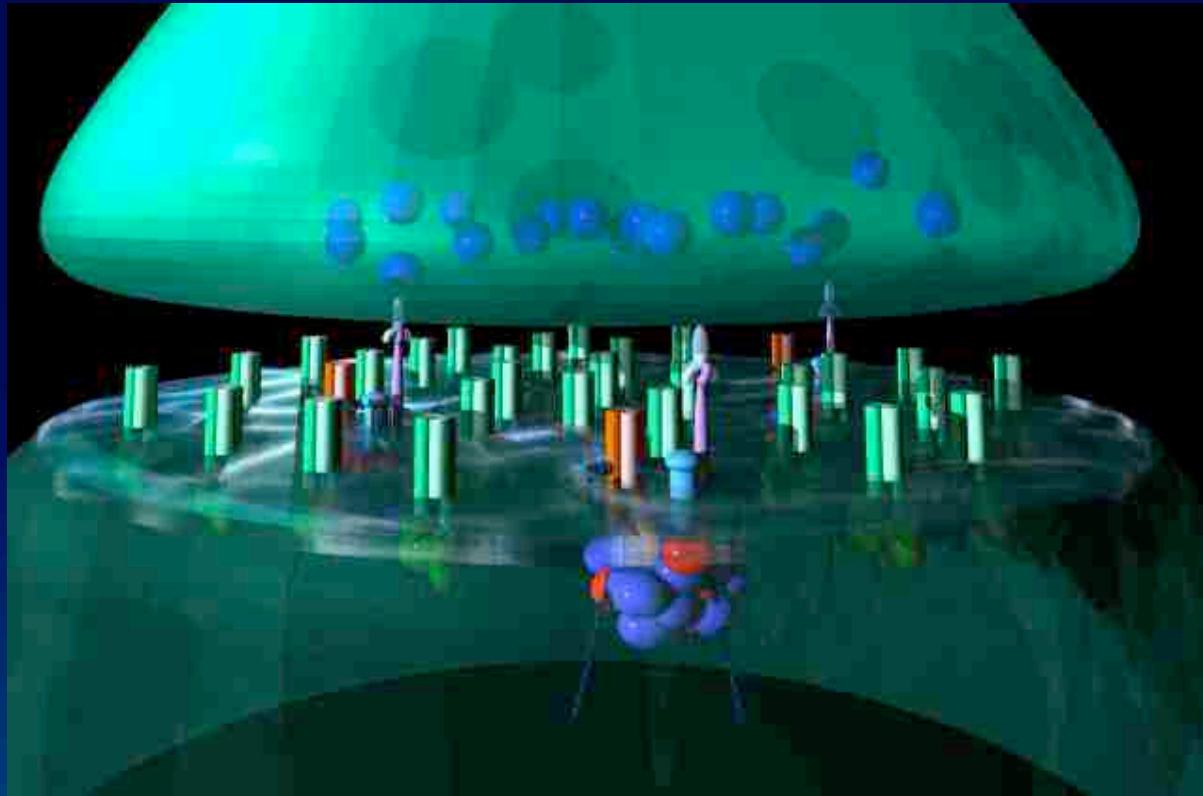


# Modelling Structure and Function of the Post-Synaptic Proteome

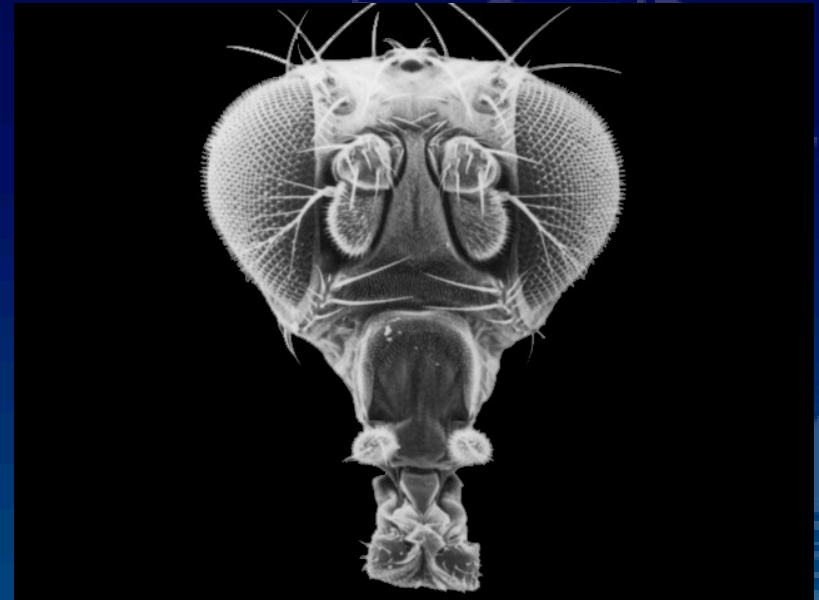


Andrew Pocklington (Edinburgh)   Seth Grant (Sanger Institute)

What can the study of simpler organisms tell us about more complex ones?



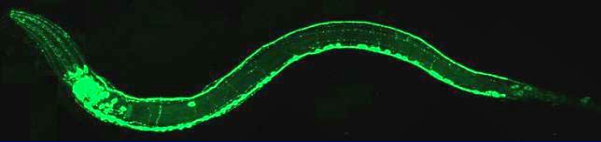
What can the study of simpler organisms tell us about more complex ones?



# Complexity – nervous system & behaviour

simple

complex



# Synapse proteomes

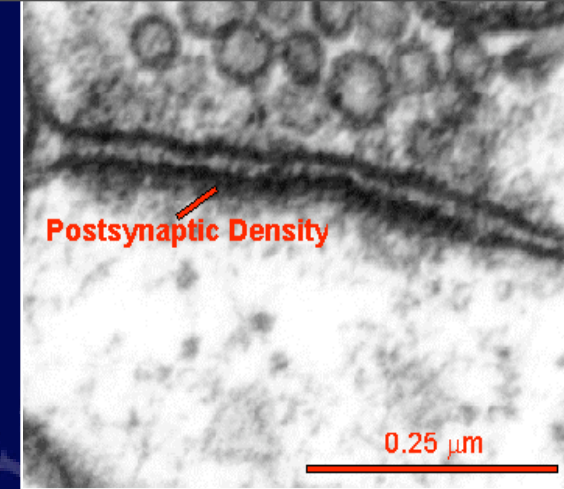
## #PSD proteins

Collins et al	620
Yoshimura et al	441
Jordan et al	401
Peng et al	328
Li et al	151
Satoh et al	46
Walikonis et al	29
Literature	119

**Total PSD 1124**

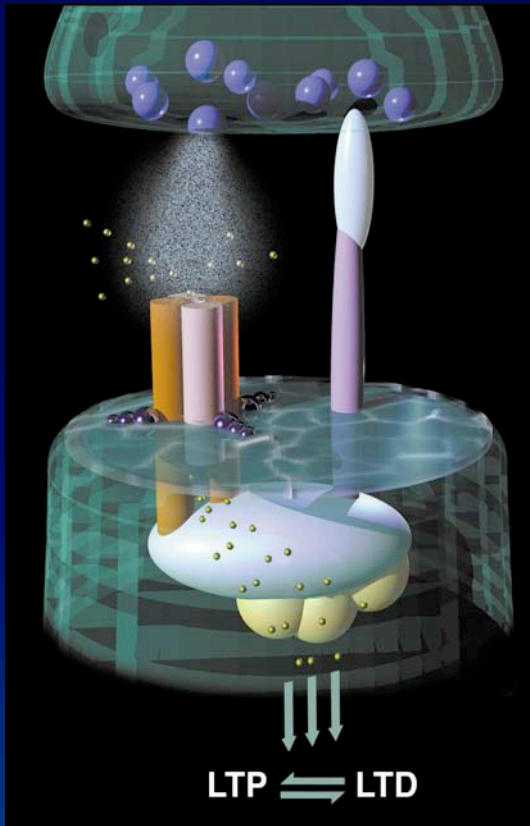
Consensus PSD 466 (2 or more studies)

<b>Total PSD proteins</b>	<b>1124</b>
<b>NRC/MASC</b>	<b>186</b>
Post Synaptic proteome	1168

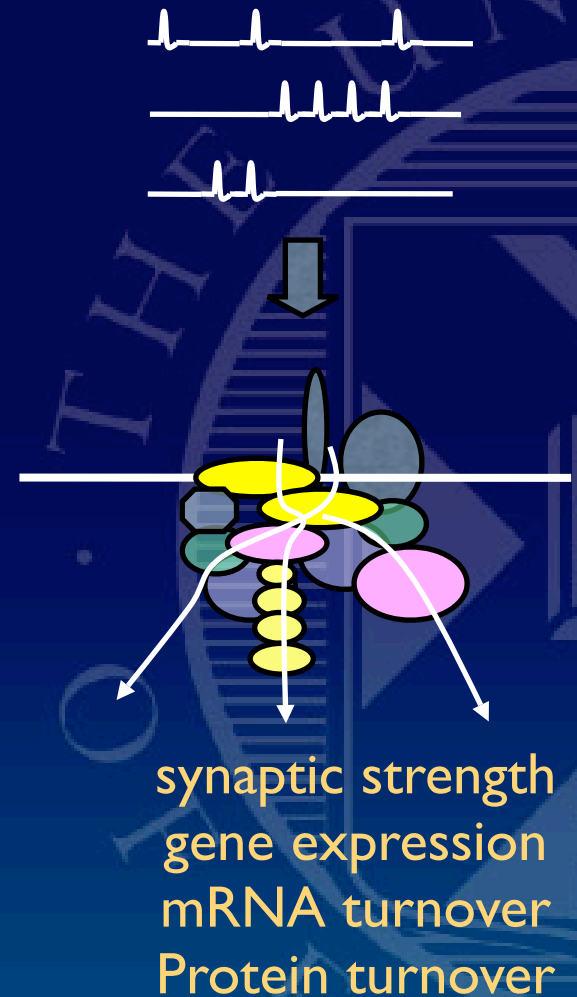




# NRC / MASC



2-3 MDa  
186 proteins



*Migaud et al, Nature, 396; 433-439. 1998;*  
*Husi et al, Nature Neuroscience, 2000*

Behaviours  
Cognition & plasticity

# 19 Species, 4 Phyla, 1.5 Billion years

Origin on 186 mouse proteins  
Orthologues in latest Ensemble release

186	<i>H. sapiens</i>	90	<i>D. melanogaster</i>
180	<i>R. norvegicus</i>	86	<i>A. gambiae</i>
165	<i>G. gallus</i>	87	<i>C. elegans</i>
177	<i>T. rubripes</i>	86	<i>C. biggsae</i>
152	<i>D. rerio</i>	60	<i>S. cerevisiae</i>

Also Chimpanzee, Rhesus, Dog, Cow, Opossum, Xenopus, Ciona & Bee  
and across all 681 PSD proteins in larger complex...

Richard Emes



# Non-Sequence Annotation

- Clinical:
  - Schizophrenia, Mental Retardation, Bipolar Disorder, Depression
- Model Organisms:
  - Rodent behaviour
  - Rodent electrophysiology: LTP/LTD.
- *Text mining*

# Annotation of MASC proteins

Schizophrenia	33
Bipolar disorder	12
Depression	14
Mental retardation	23
LTP	44
Rodent spatial learning	32
Rodent fear conditioning	25

(186)

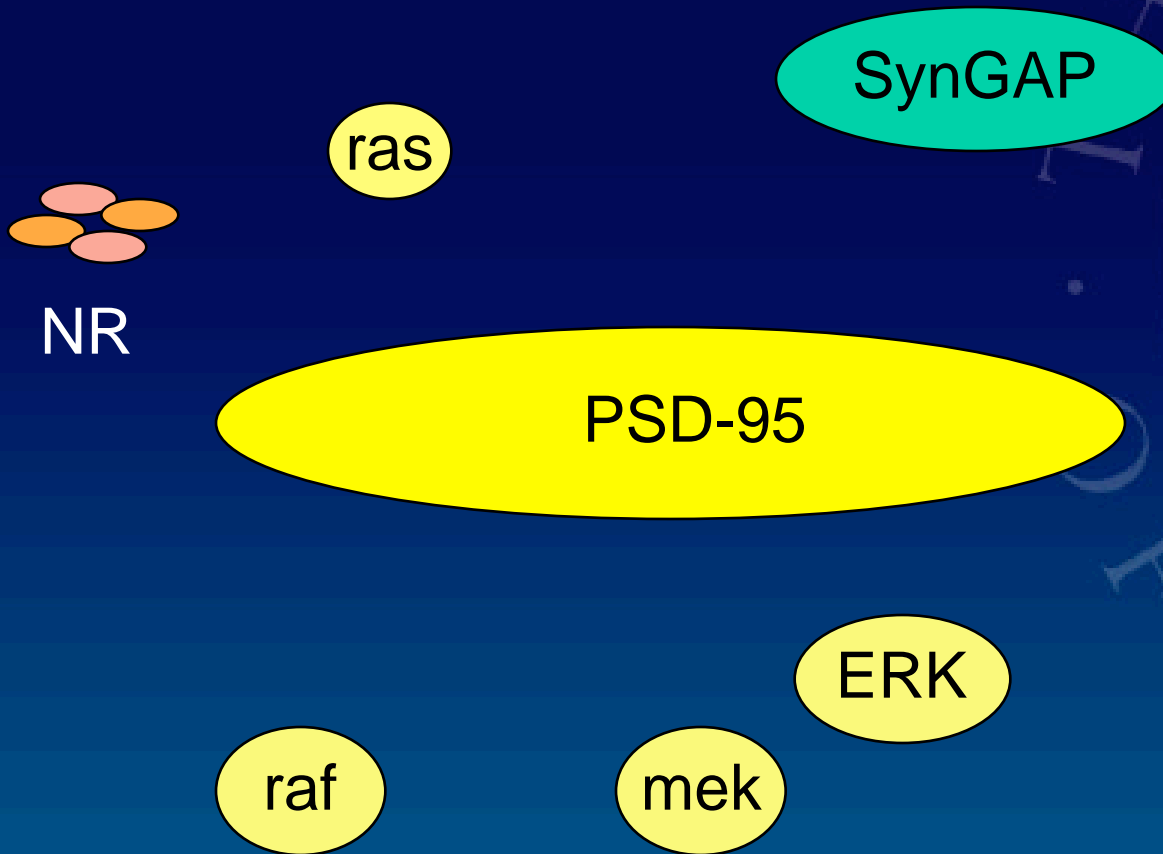
# Annotation of MASC proteins

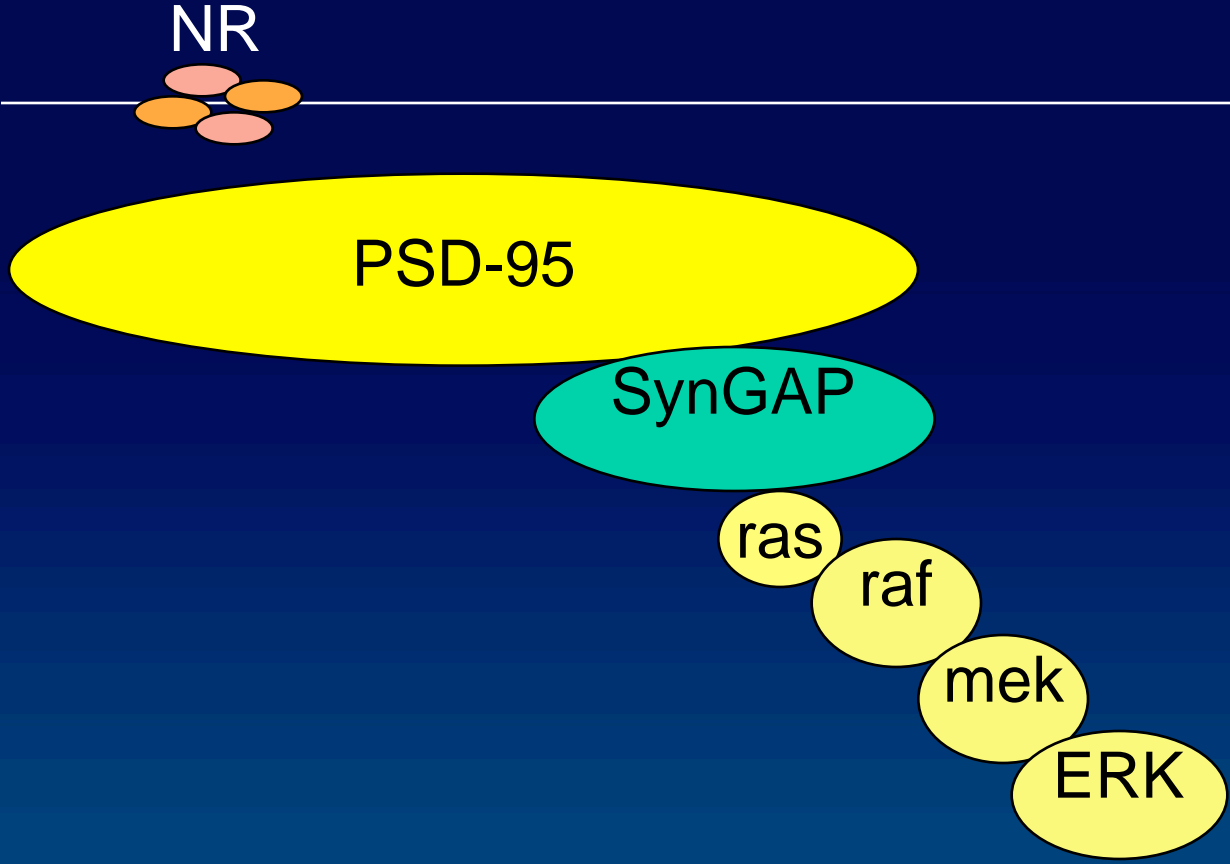
		expect (random)
Schizophrenia	33	3
Bipolar disorder	12	
Depression	14	
Mental retardation	23	
LTP	44	3
Rodent spatial learning	32	2
Rodent fear conditioning	25	1

(186)

# Need to rebuild the network from the proteomics derived list

---





'Ras-MAPK pathway'



# Text Mining

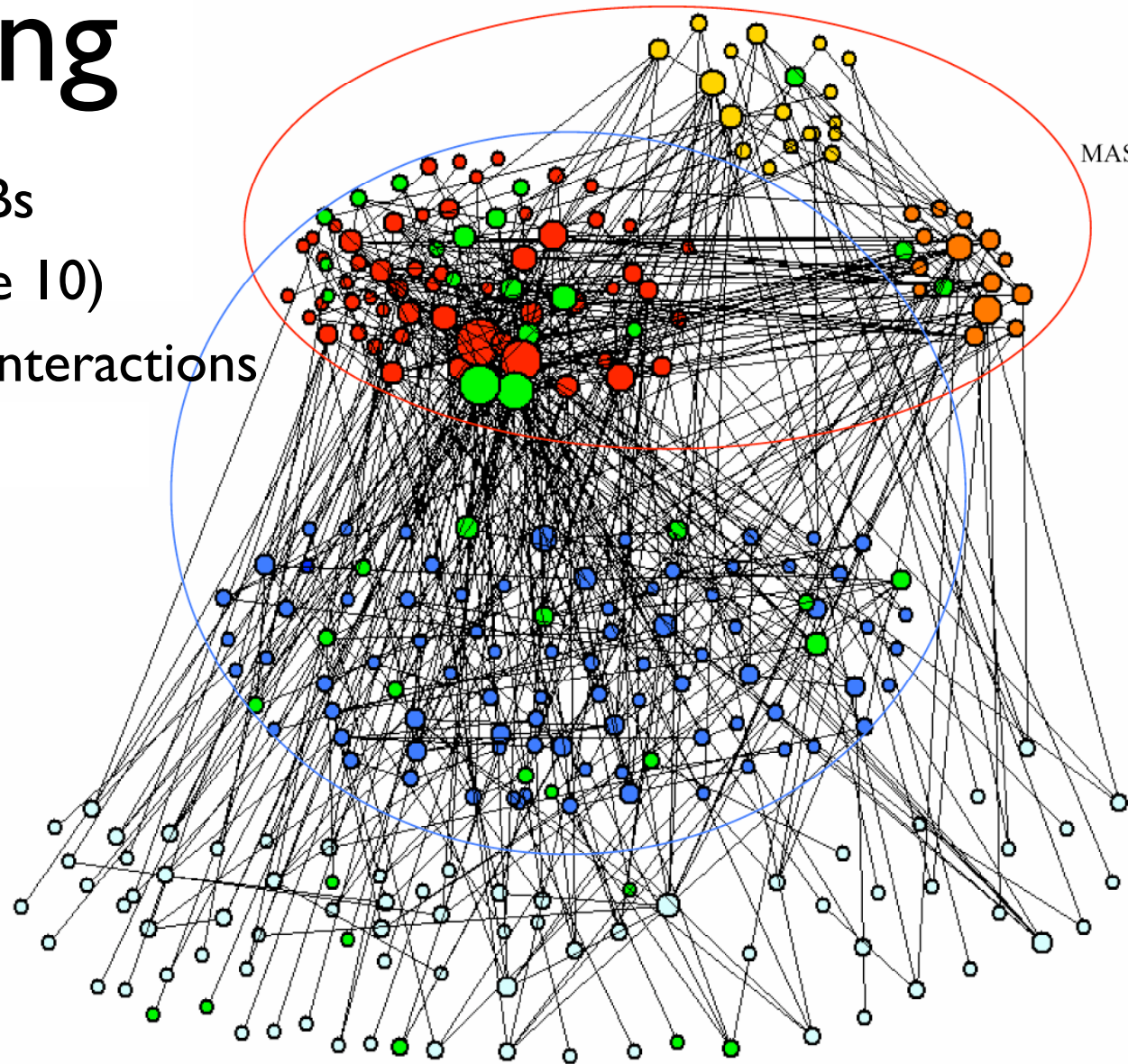
Start with existing DBs

Find all synonyms (ave 10)

REGEX patterns for interactions

Manual Curation

Checked twice



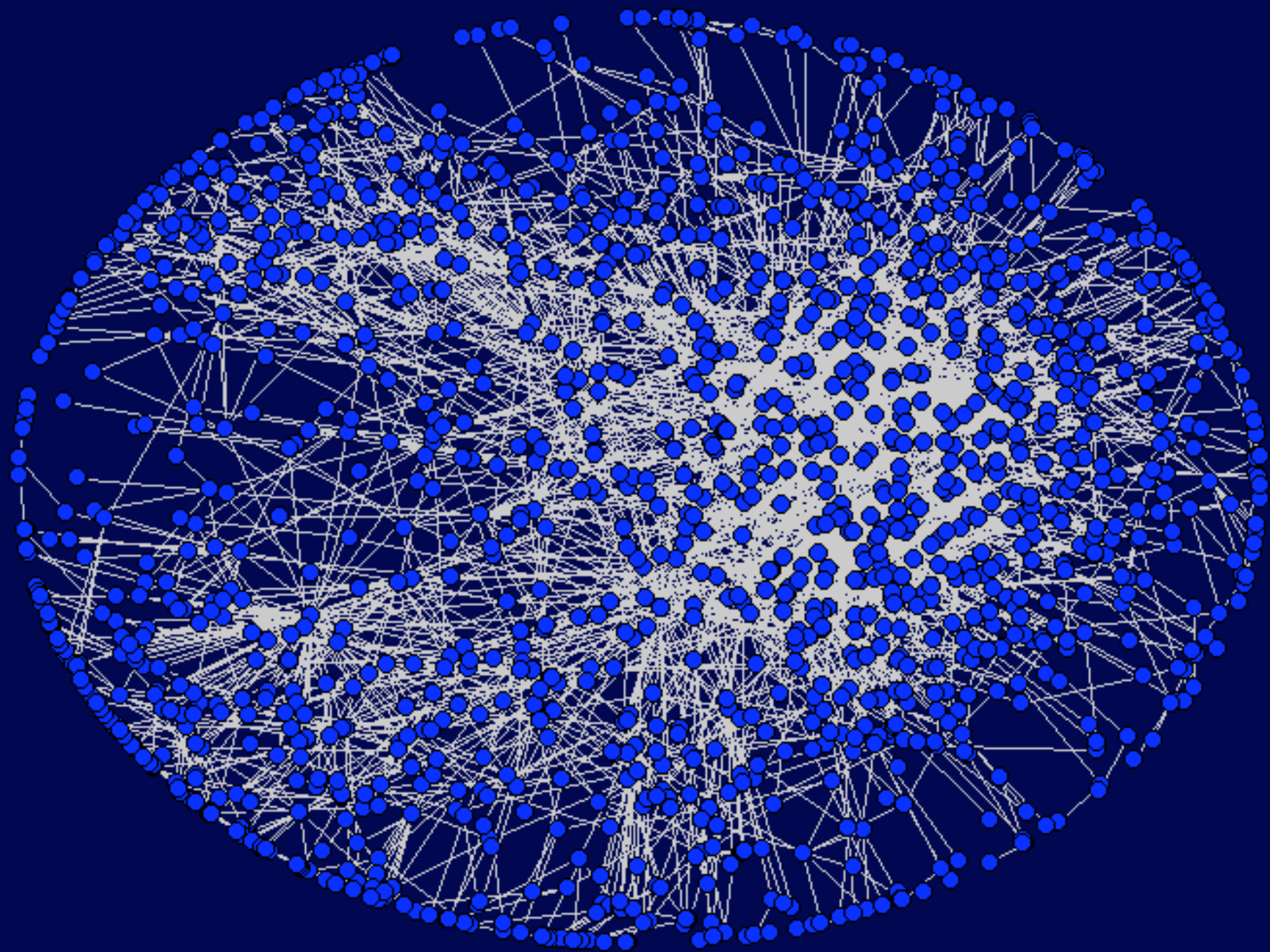
Mark Cumiskey, Keri  
Page & Mike Marshall  
[www.ppid.org](http://www.ppid.org)

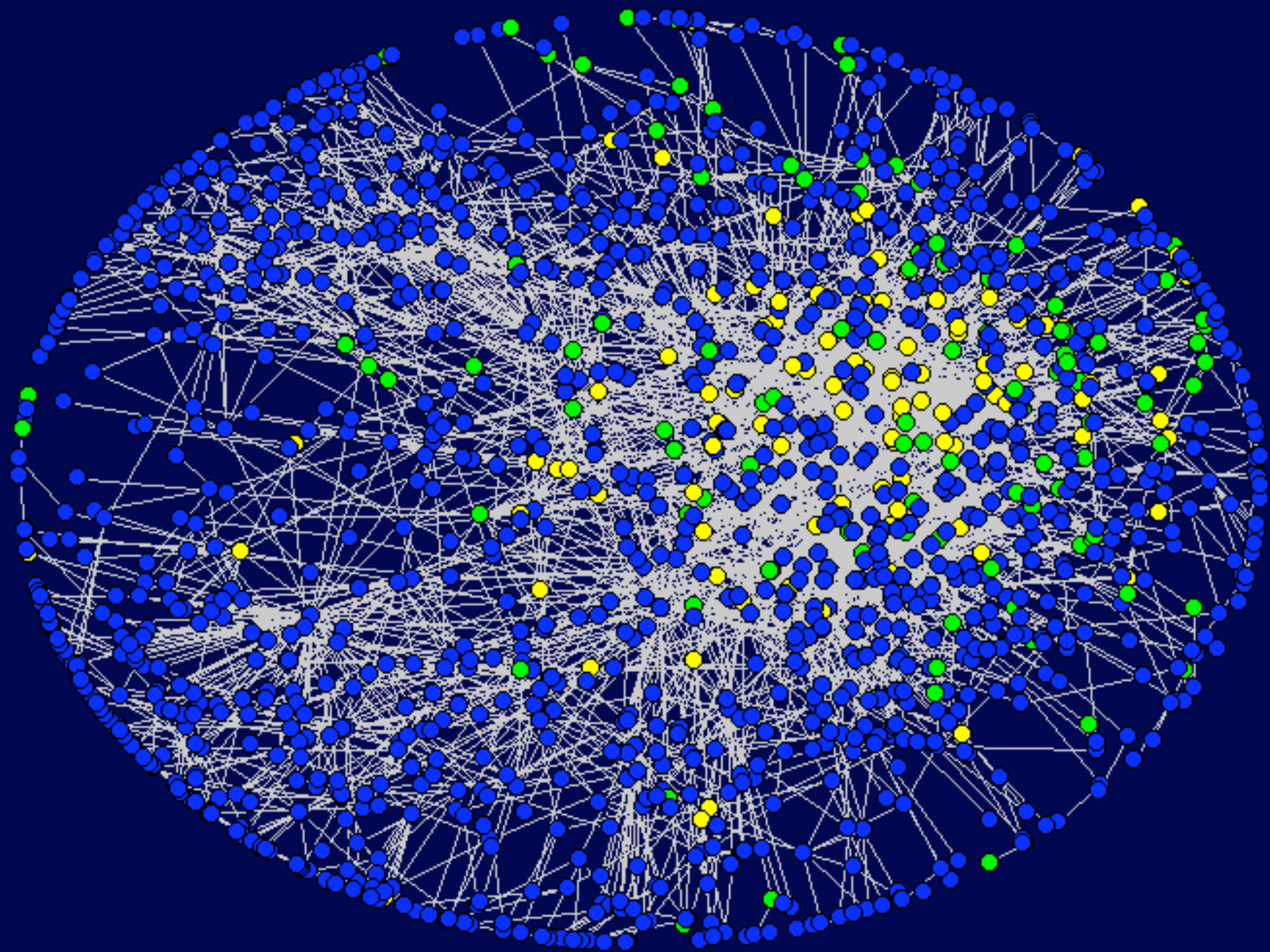


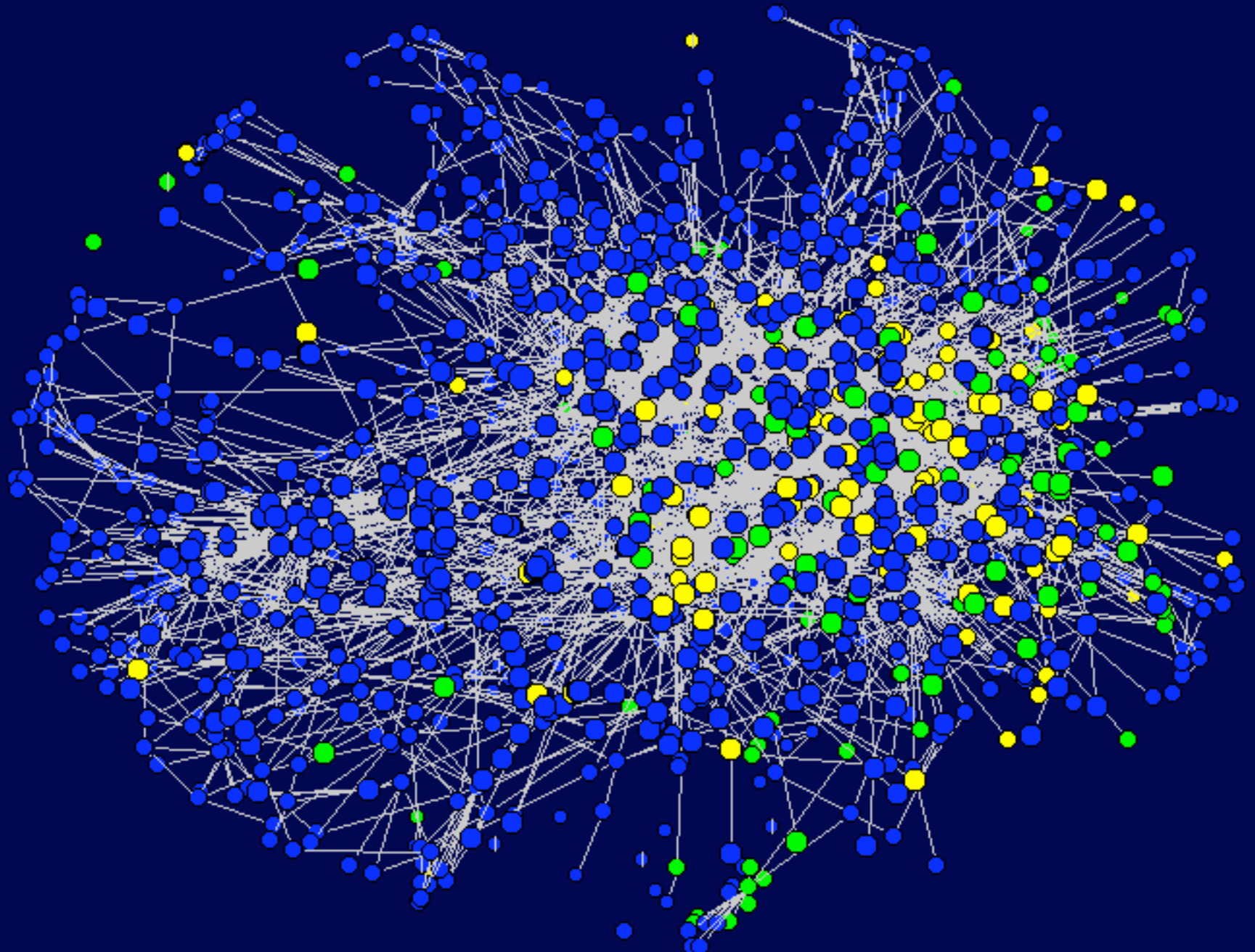










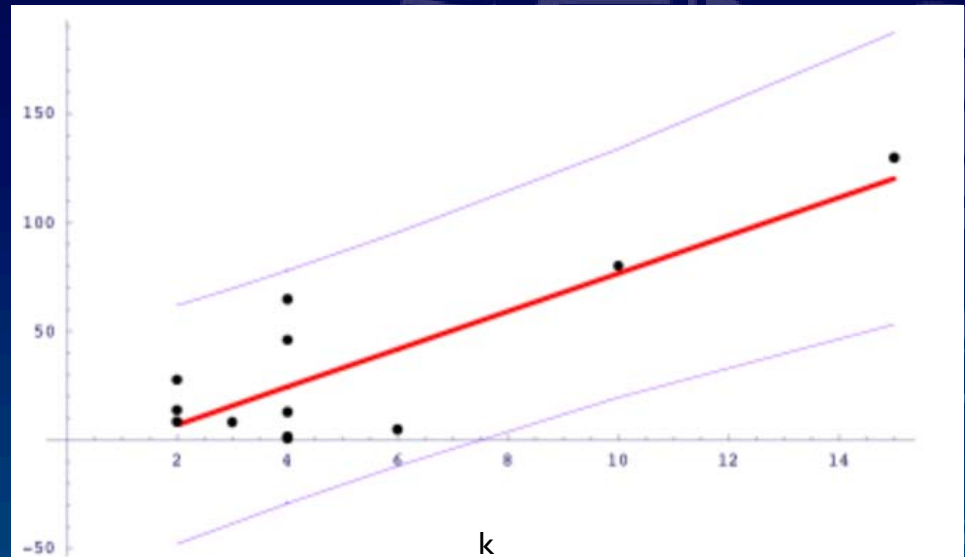




# Simulated disruption vs. mutations

100Hz LTP data crated  
from literature.

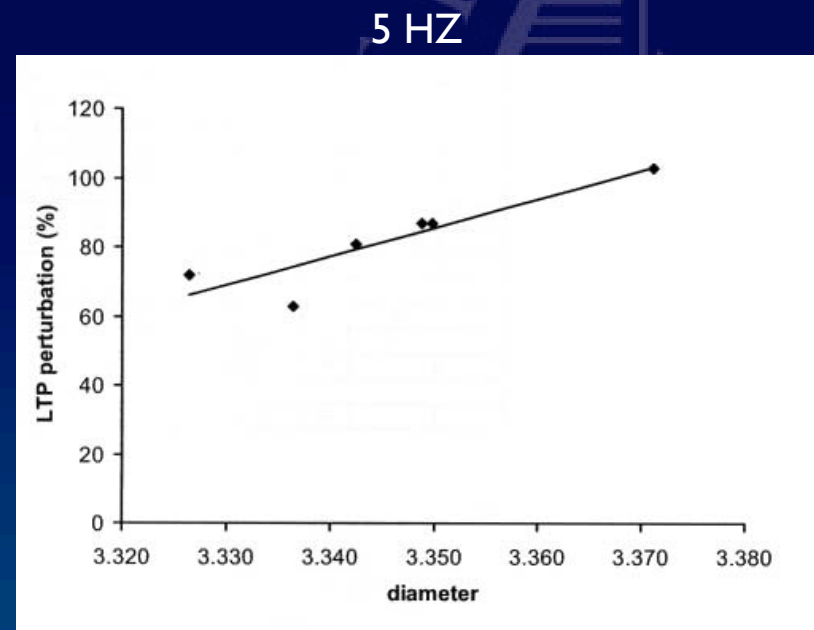
Linear correlation  
between simulation and *in vivo*  
assay. ( $p < 0.01$ )



# Simulated disruption vs. mutations

Linear correlation between simulation and *in vivo* assay

Details: Mutations in MEK1, SynGAP, NR2AC, PKA, PI3-kinase, PSD-95 were all analysed in a single laboratory (TJ O'Dell, UCLA) under controlled conditions and LTP disruption measured. ( $p < 0.05$ )



H. Husi J. Choudhary L. Yu M. Cumiskey W. Blackstock T.J. O'Dell P.M. Visscher J.D. Armstrong S.G.N. Grant, unpublished

# so far, so good?

- Small scale static network model
- Explains cross-talk and redundancy
- *Does not capture dynamic processes*
- *Does not capture changes across the brain.*

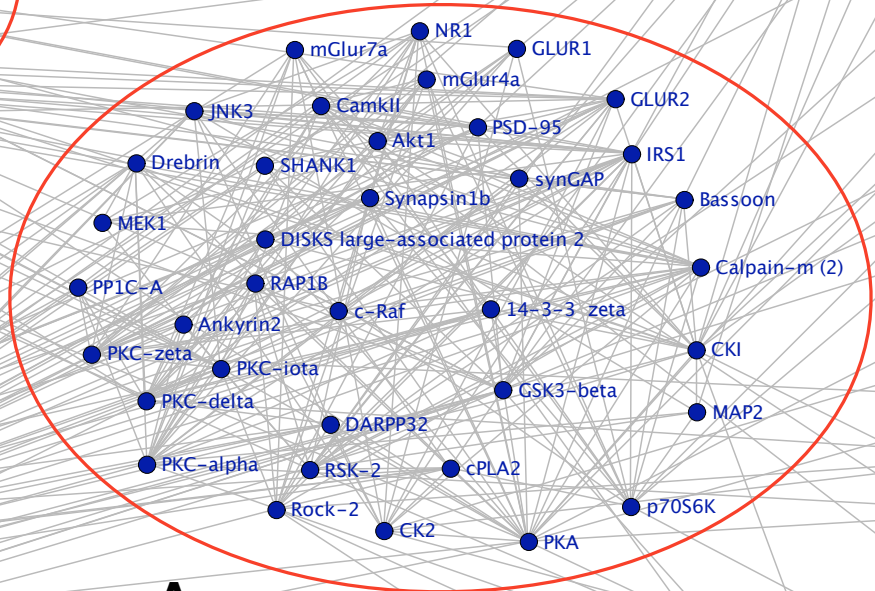
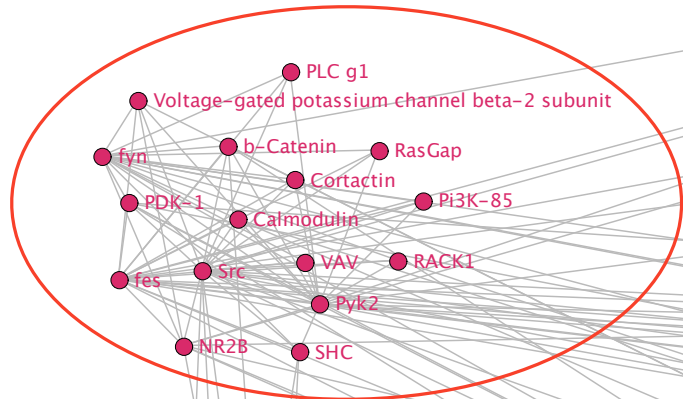




# regulation/dynamics

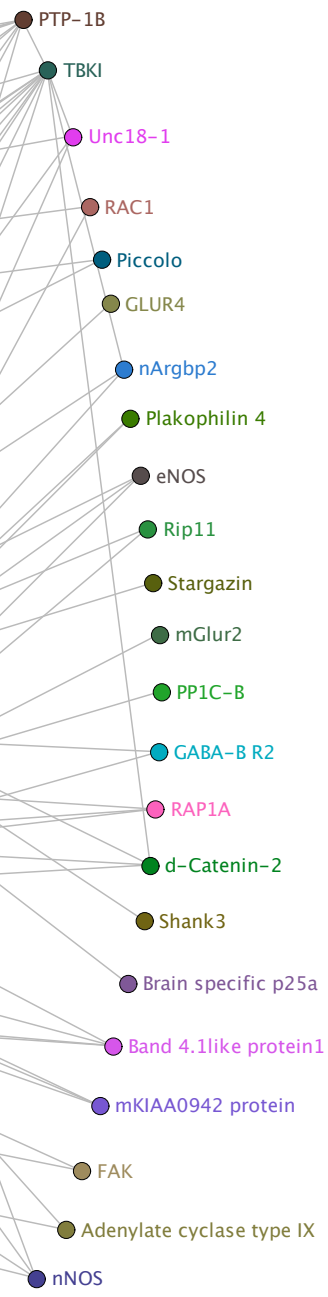
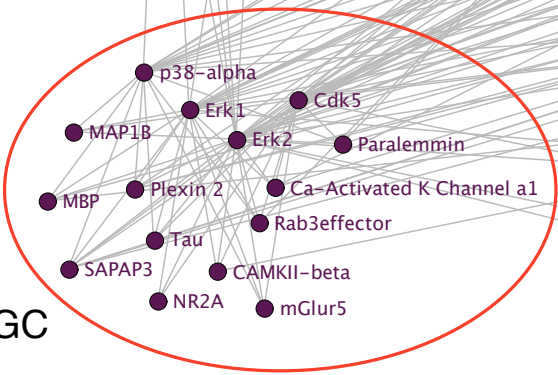
- Good models of a few pathways
- 25 kinases
- 600 potential phosphorylation sites in PSP
- phospho-peptide array

**C**  
 PTK  
 SH2 ligand  
 AP (endocytosis) signalling

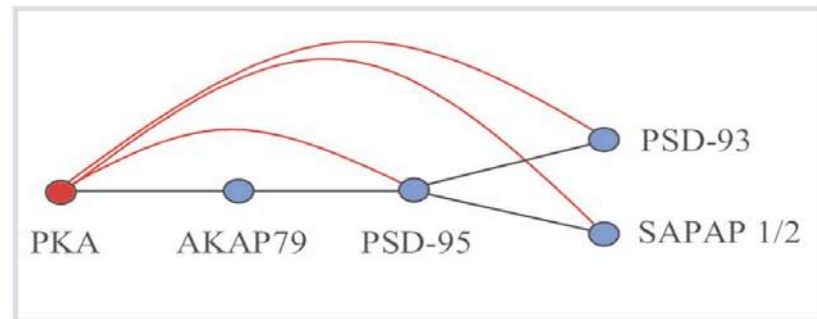
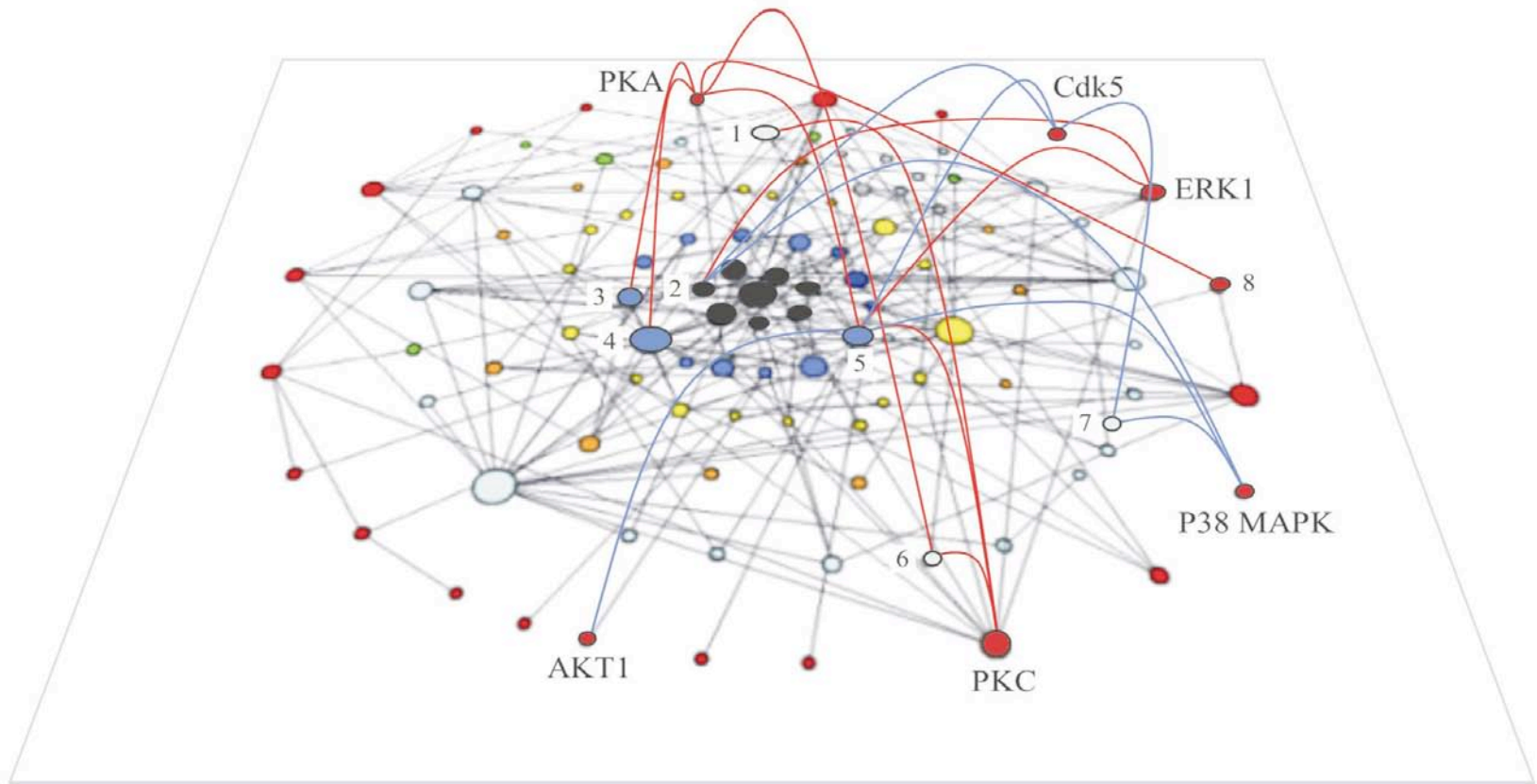


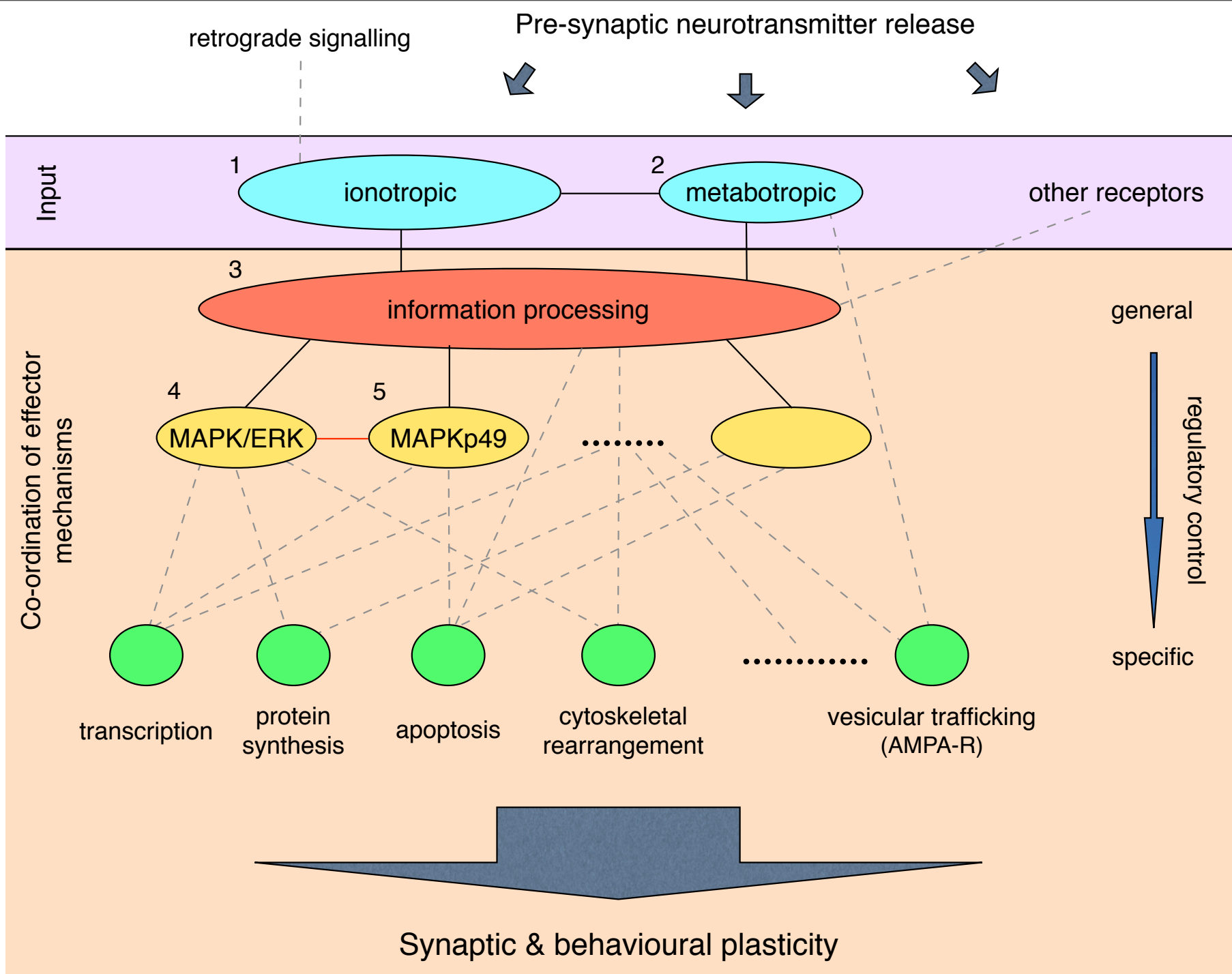
**A**  
 AGC  
 Glutamate receptors  
 2nd messengers (Ca, cAMP, DAG)

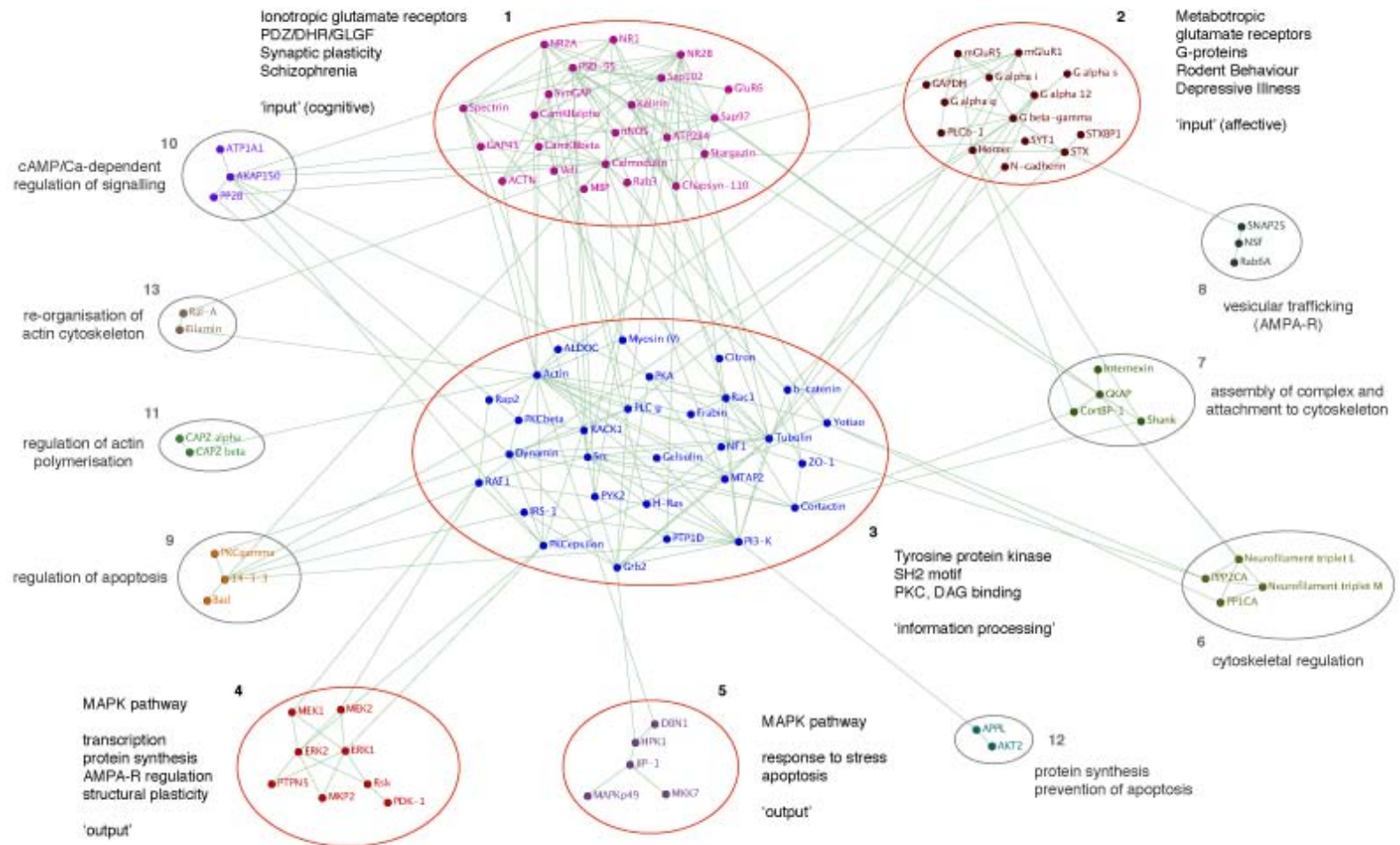
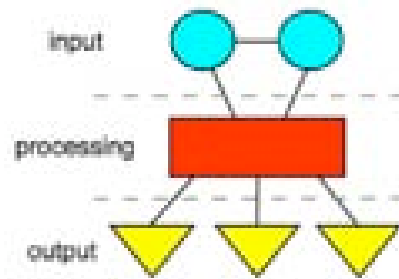
**B**  
 CMGC  
 p35  
 modulators  
 WW ligand  
 SH3 ligand

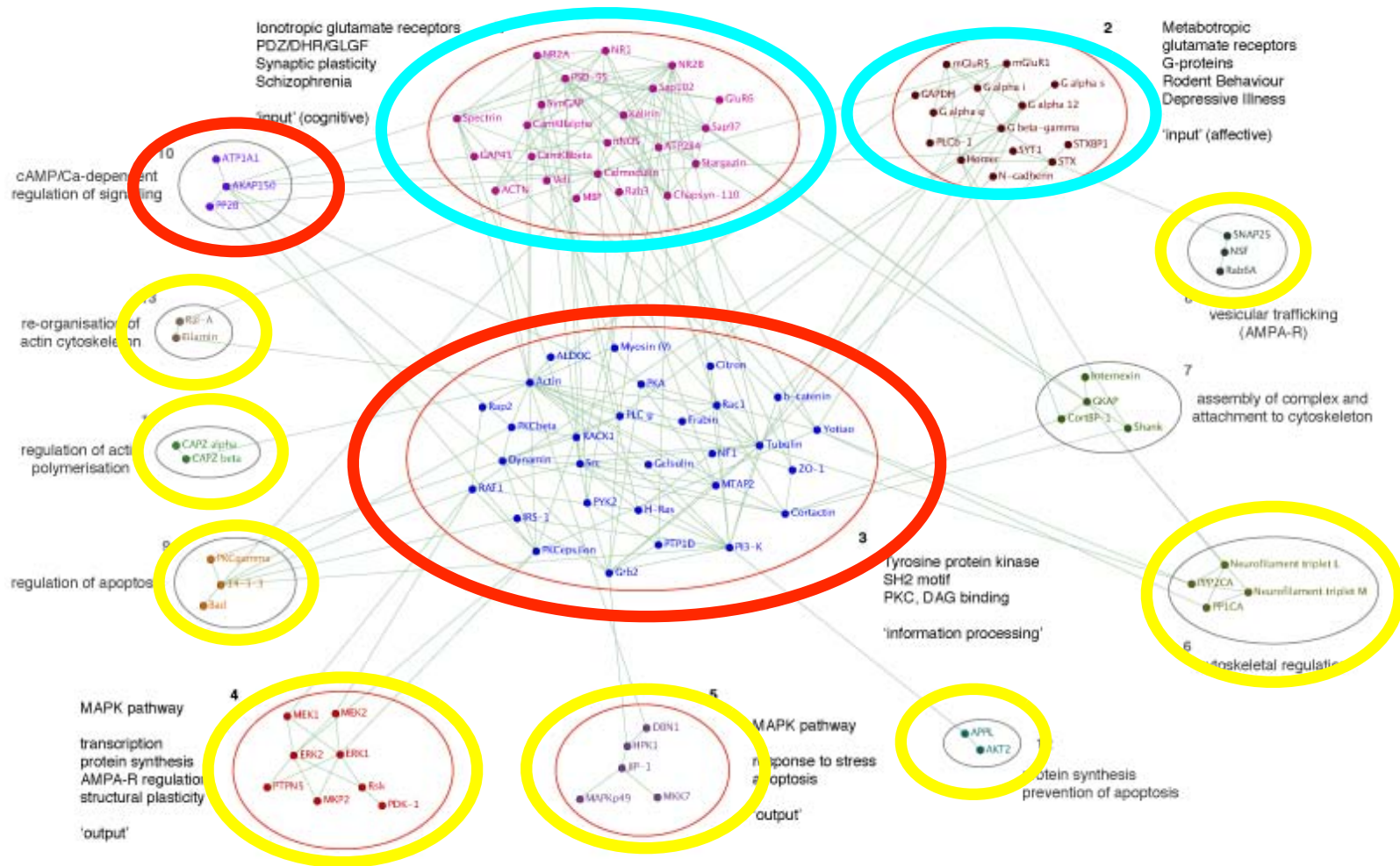
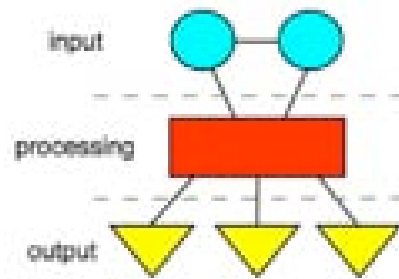


# phosphorylation in the complex

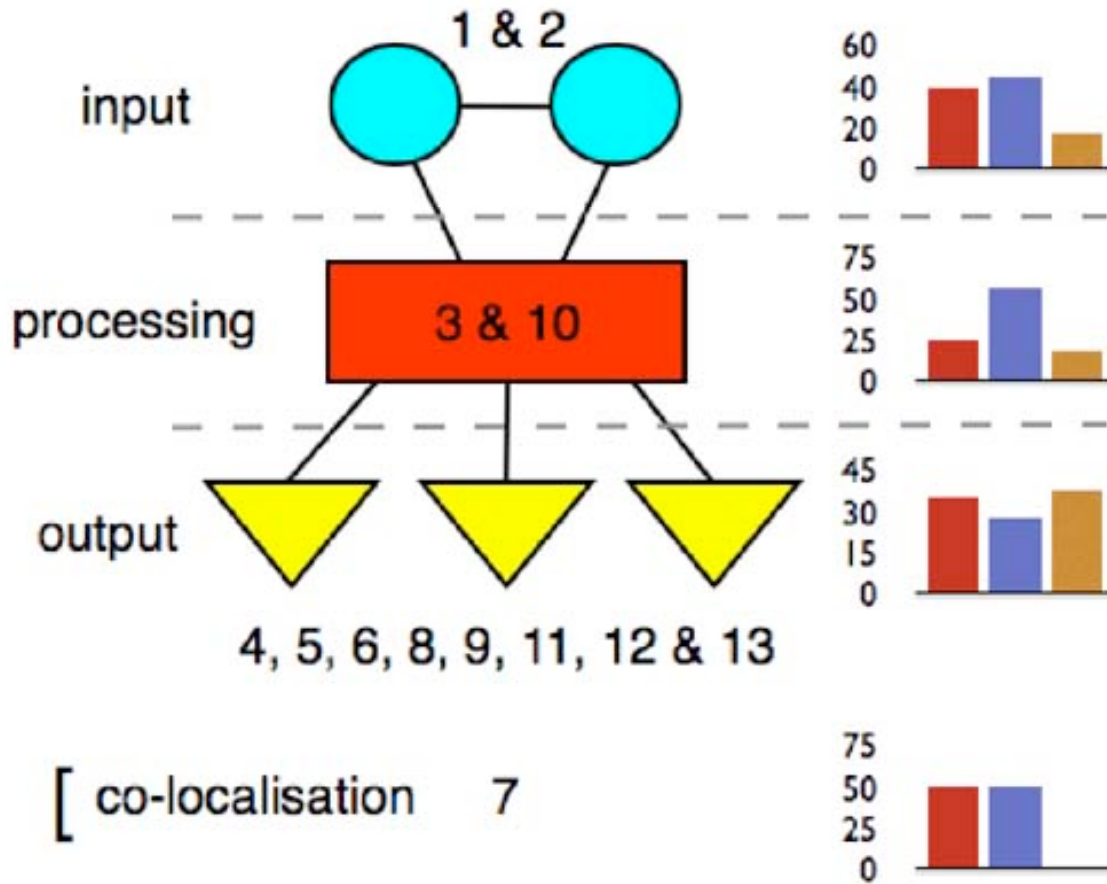








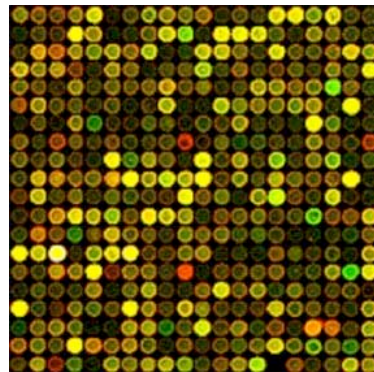
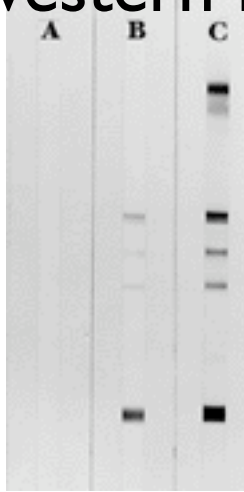
# evolutionary origin



# what does the complex look like across the brain?

Chris Anderson

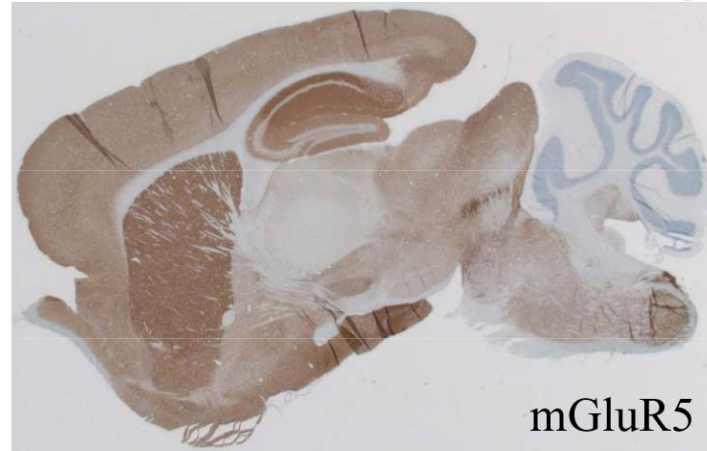
## Western Blot



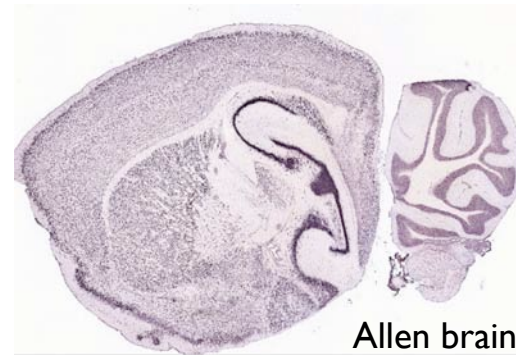
St Jude Brain Gene Expression Map

## Microarray

## Immunohistochemistry



protein

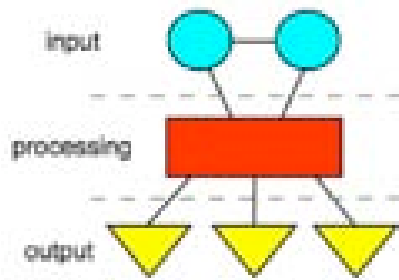


mRNA

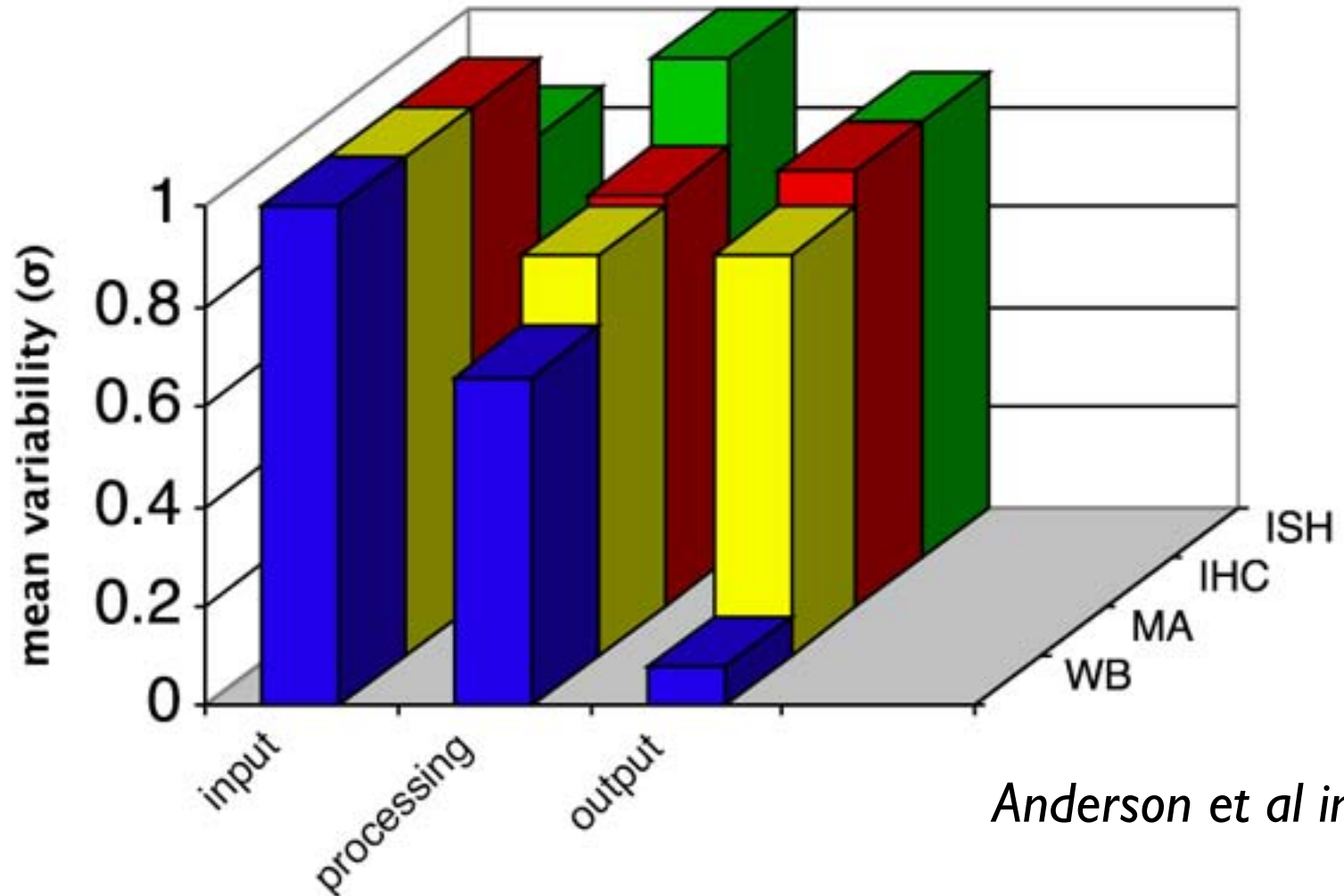
## *In situ* hybridisation

Quantitative  $\longrightarrow$  Qualitative



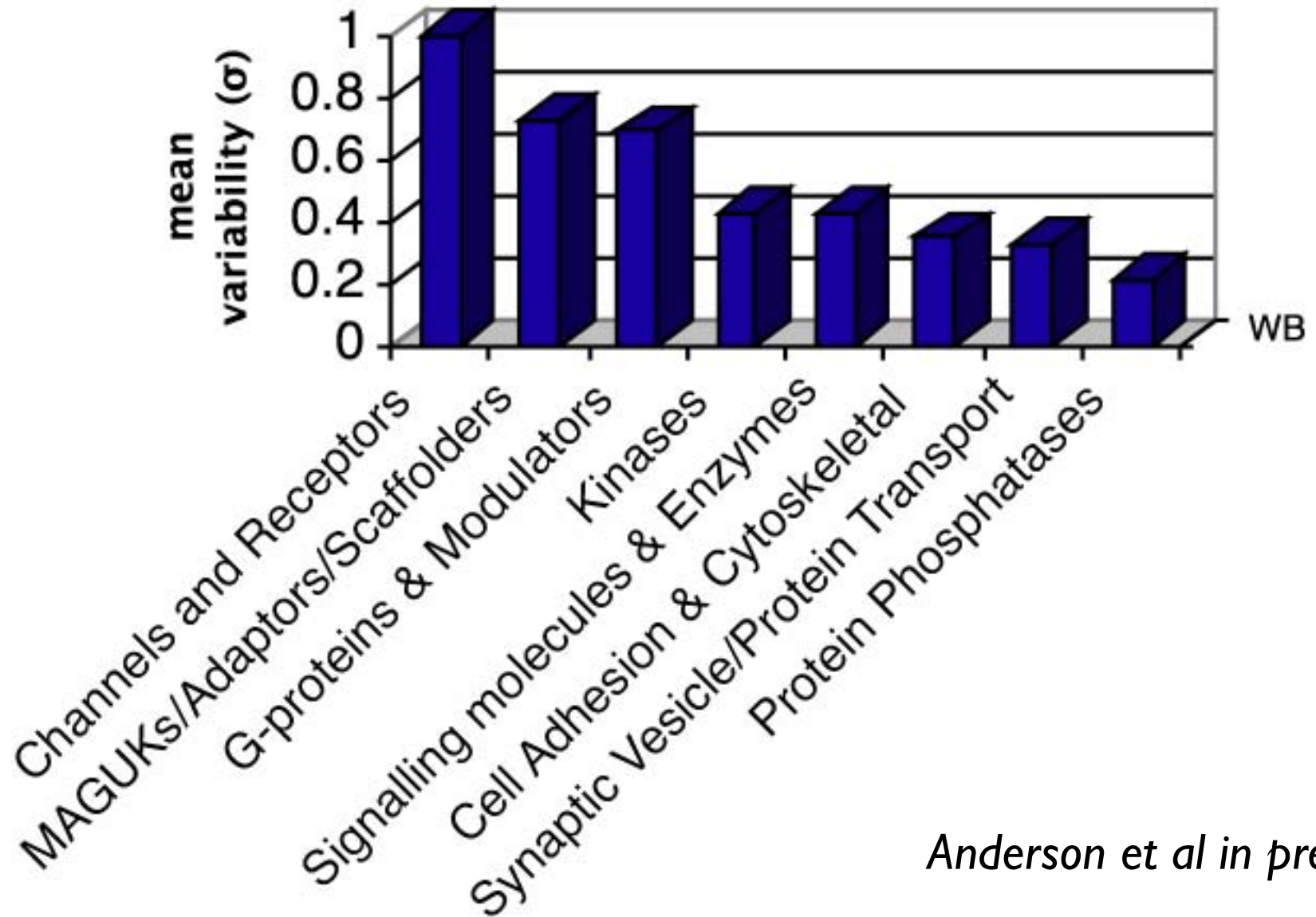


clusters with variance

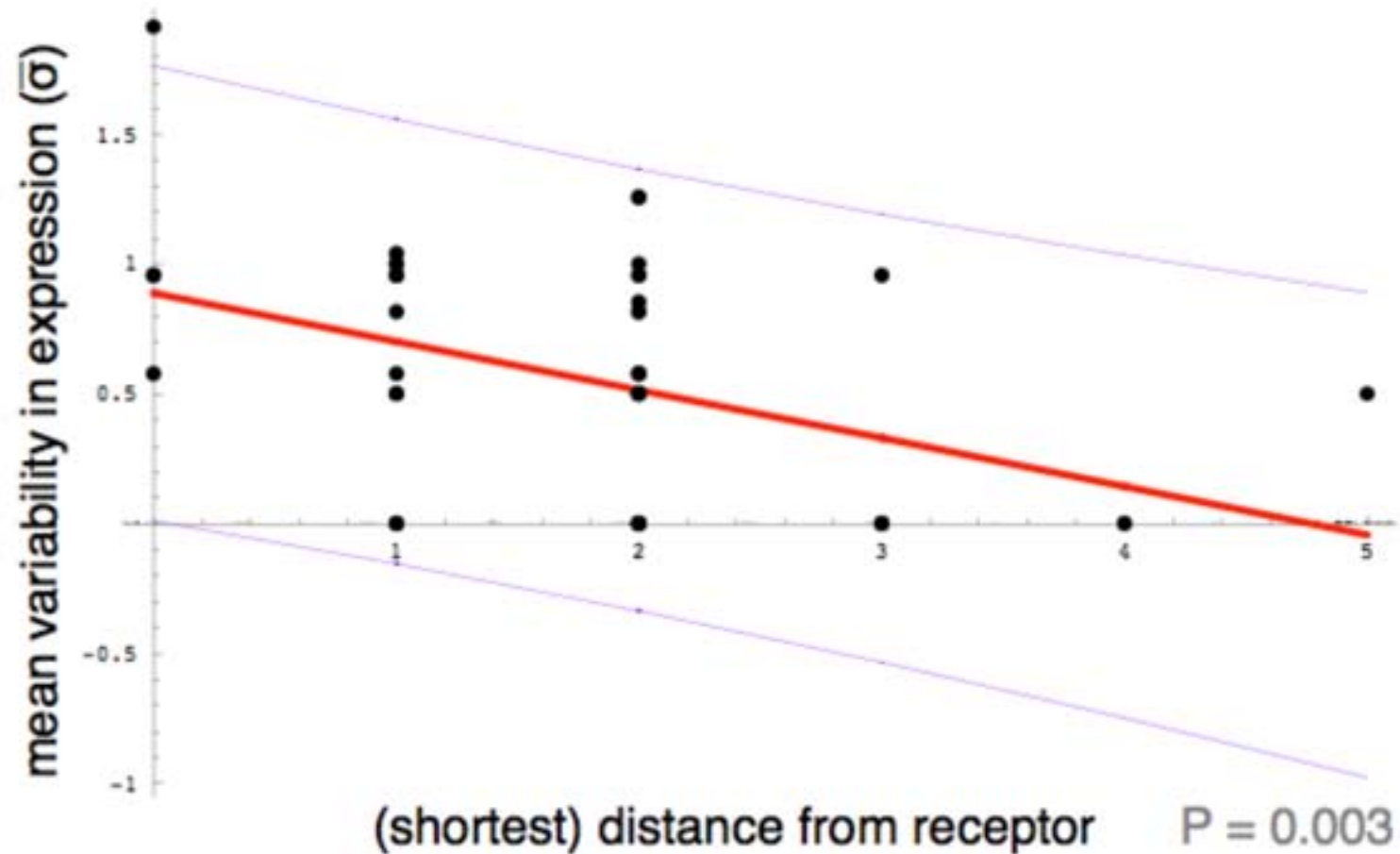


*Anderson et al in prep*

# variability with protein type

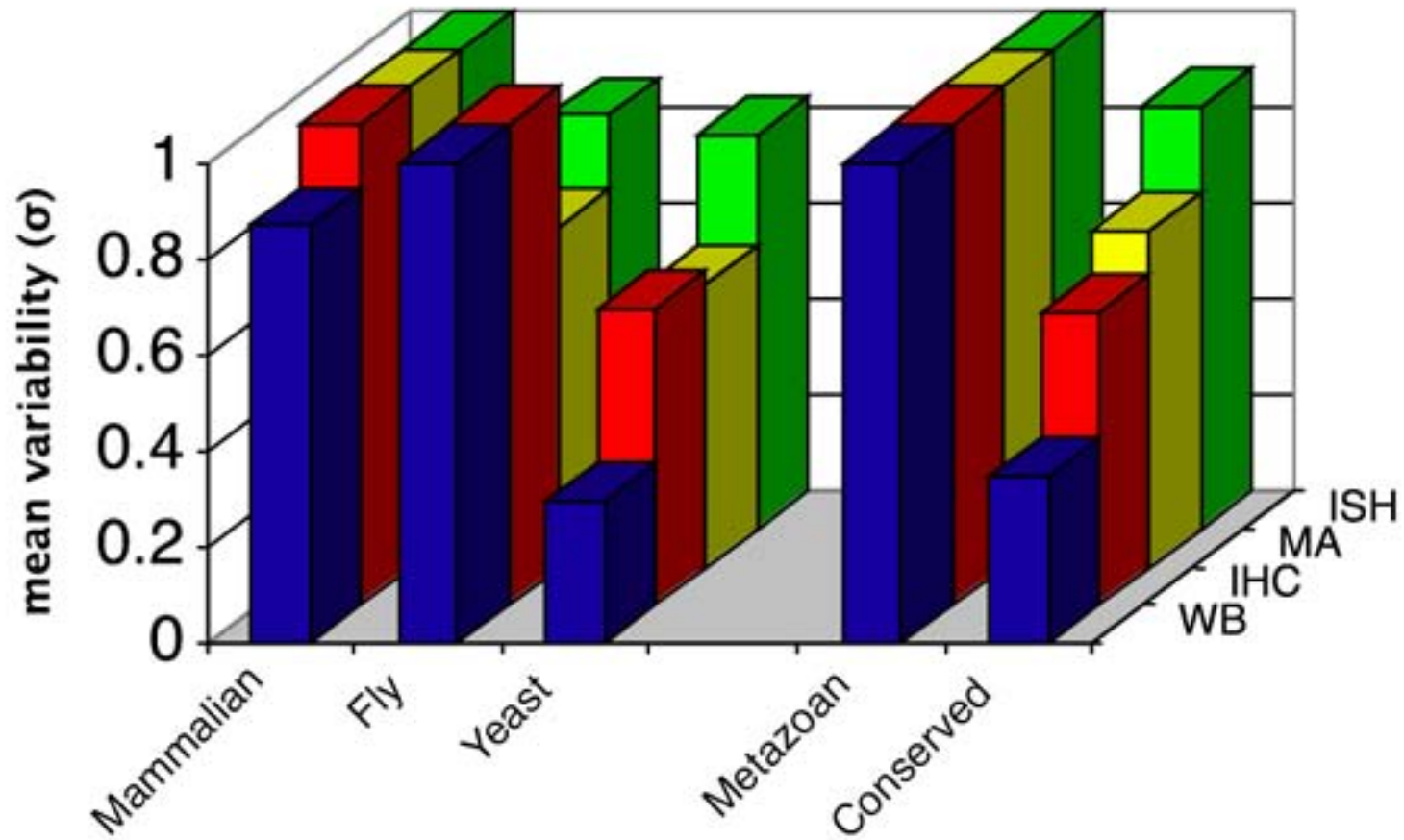


variability inversely correlates with distance from NMDA receptor



*Anderson et al in prep*

# evolutionary emergence with variance



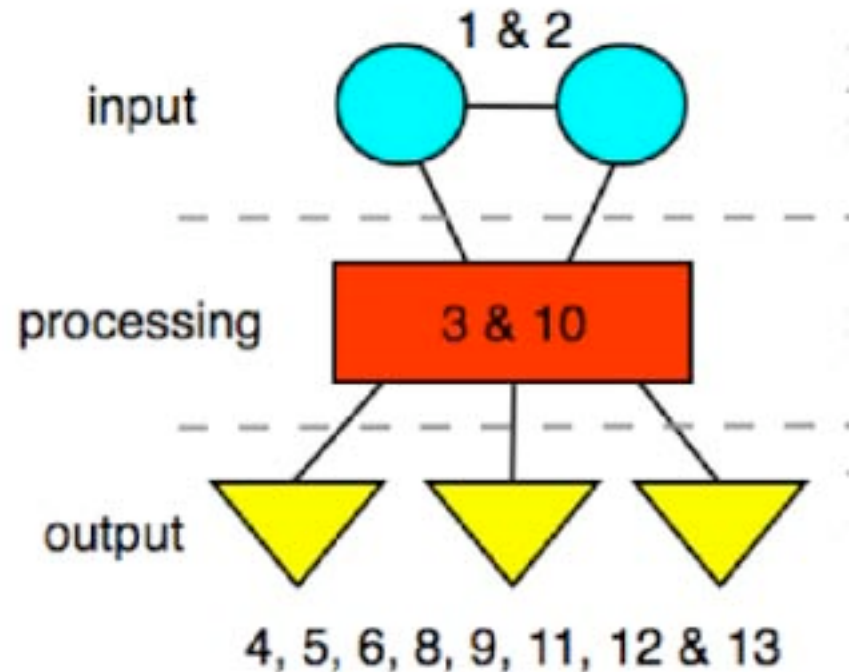
*Anderson et al in prep*

# molecular computation in fly neurons

behav	lethal
<b>67%</b>	65%
40%	75%
50%	<b>100%</b>

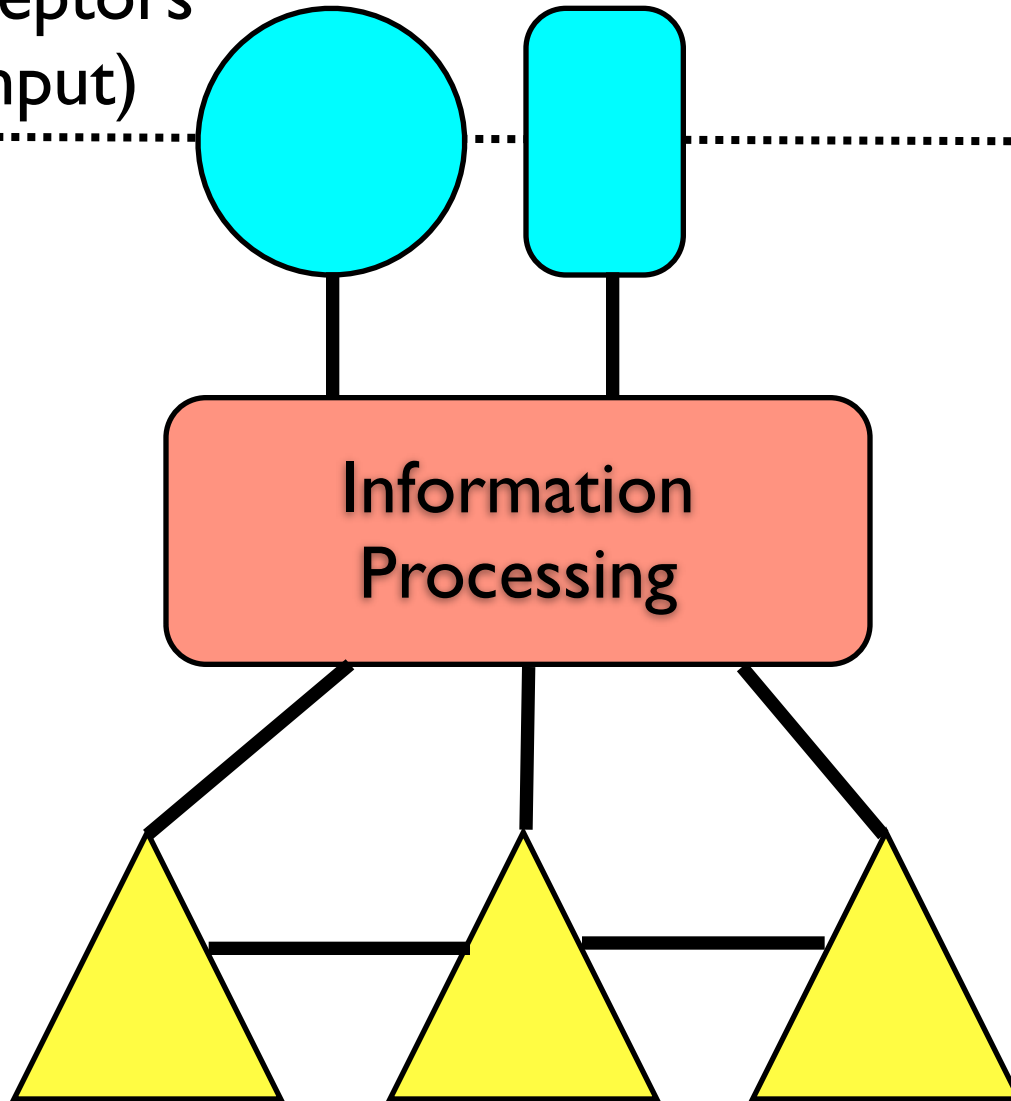
90 orthologues in fly  
1471 total alleles

Significant correlation between fly behaviour phenotype and affective ( $P < 0.004$ ) and cognitive ( $P < 0.01$ ) disorders



All main clusters and structure represented

Receptors  
(input)

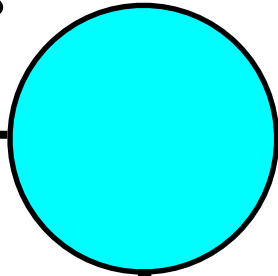


cell membrane

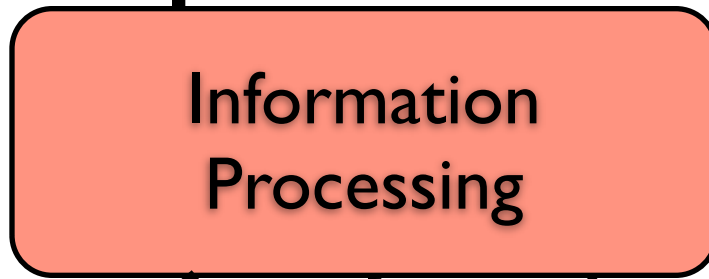
Signalling complex  
specificity at the cell  
type is defined by  
receptor  
complement

output pathways

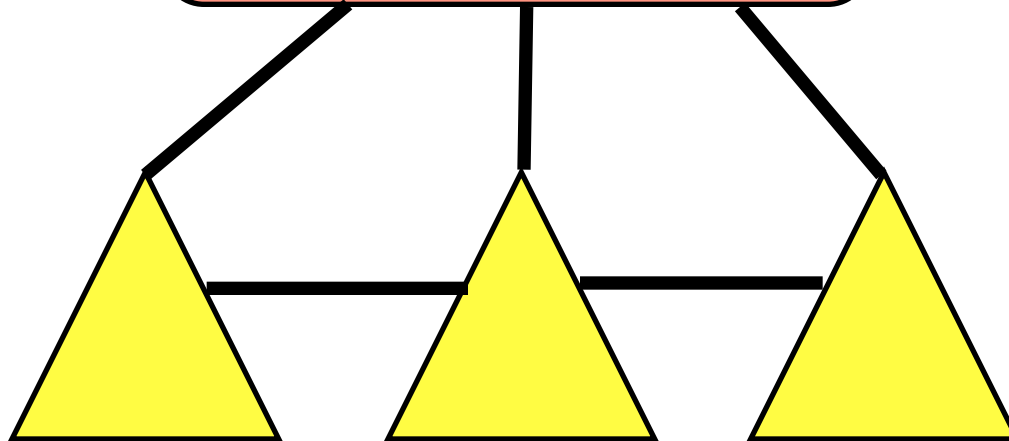
Receptors  
(input)



cell membrane



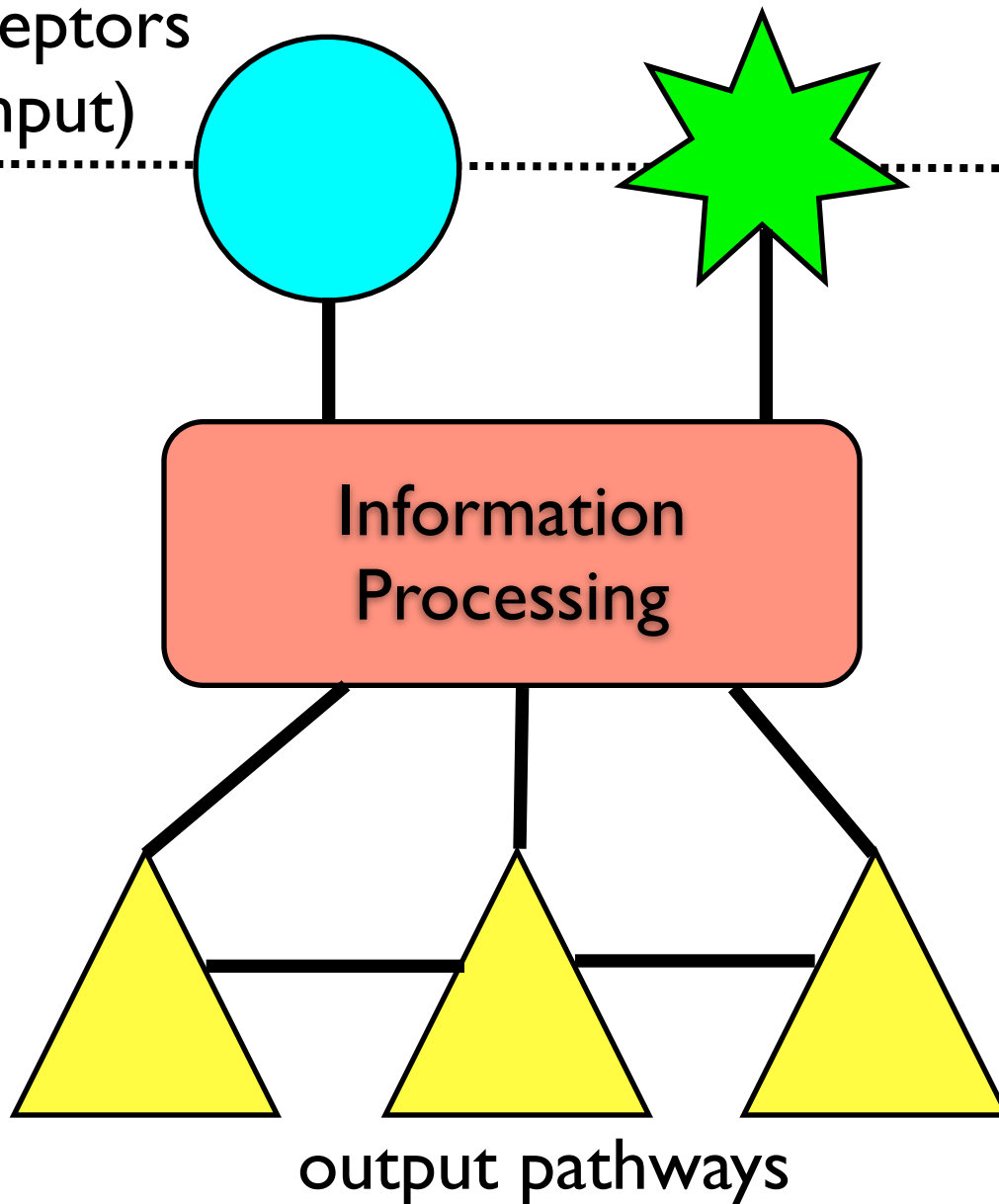
Information  
Processing



output pathways

Signalling complex  
specificity at the cell  
type is defined by  
receptor  
complement

Receptors  
(input)

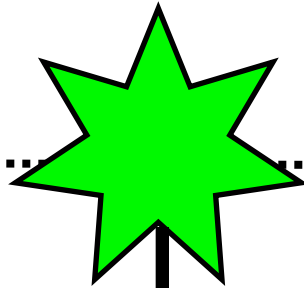
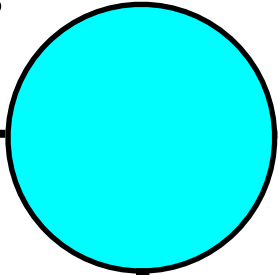


cell membrane

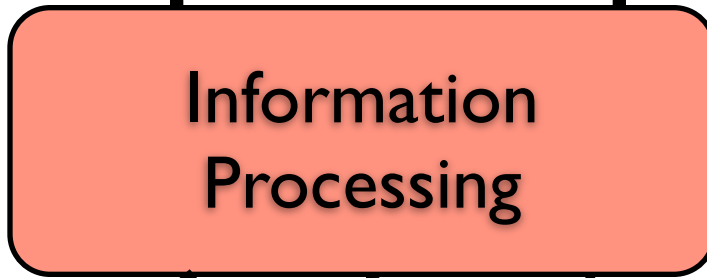
Signalling complex  
specificity at the cell  
type is defined by  
receptor  
complement



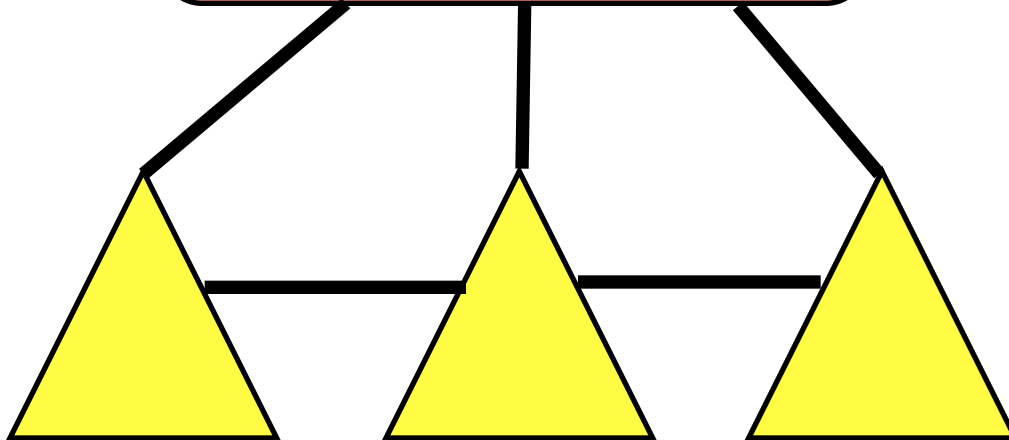
Receptors  
(input)



cell membrane

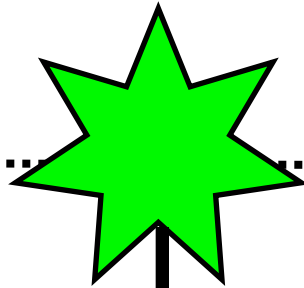
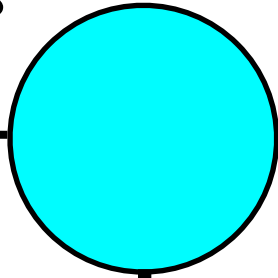


Information  
Processing

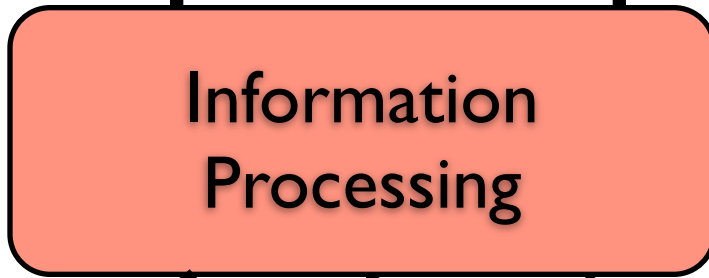


output pathways

Receptors  
(input)

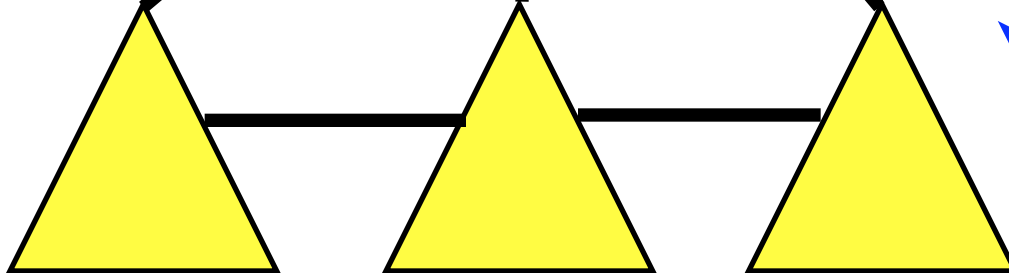


cell membrane



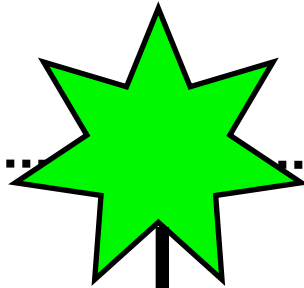
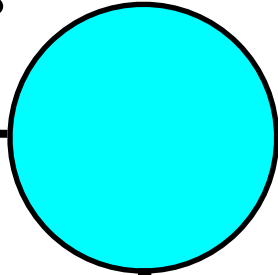
Information  
Processing

Phosphorylative  
regulation of common  
pathways is top down

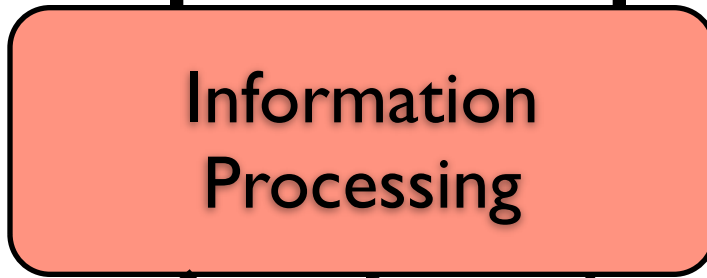


output pathways

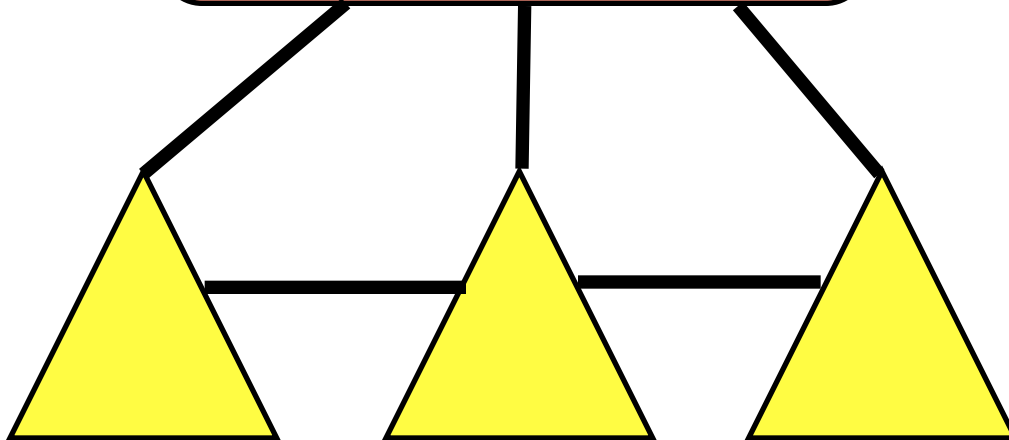
Receptors  
(input)



cell membrane

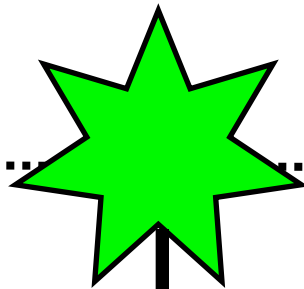
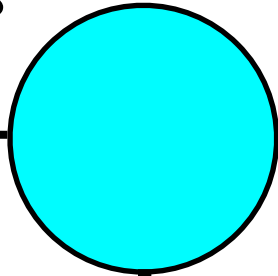


Information  
Processing

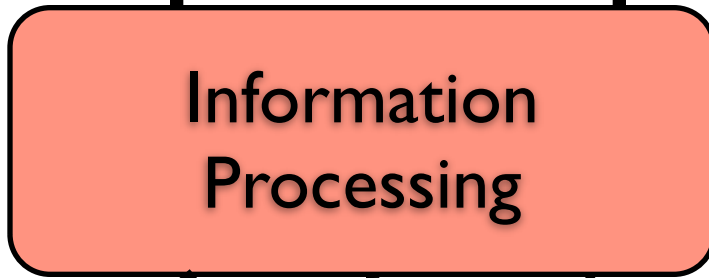


output pathways

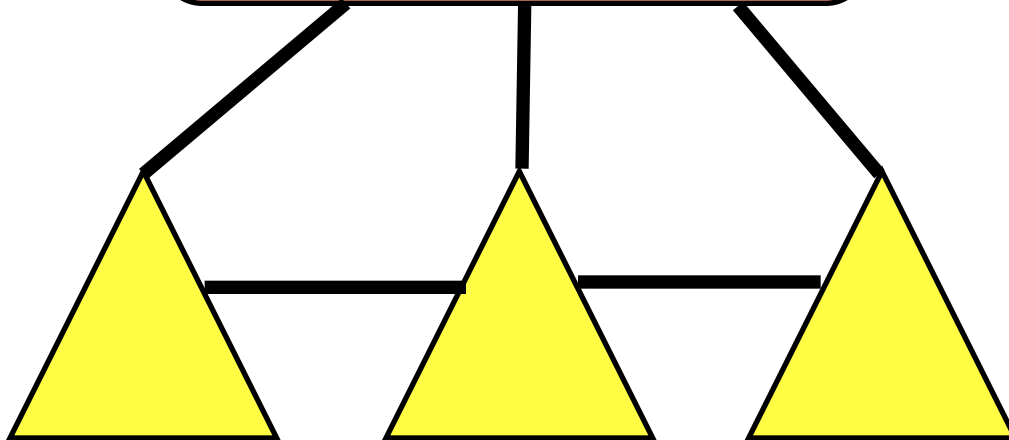
Receptors  
(input)



cell membrane



Information  
Processing

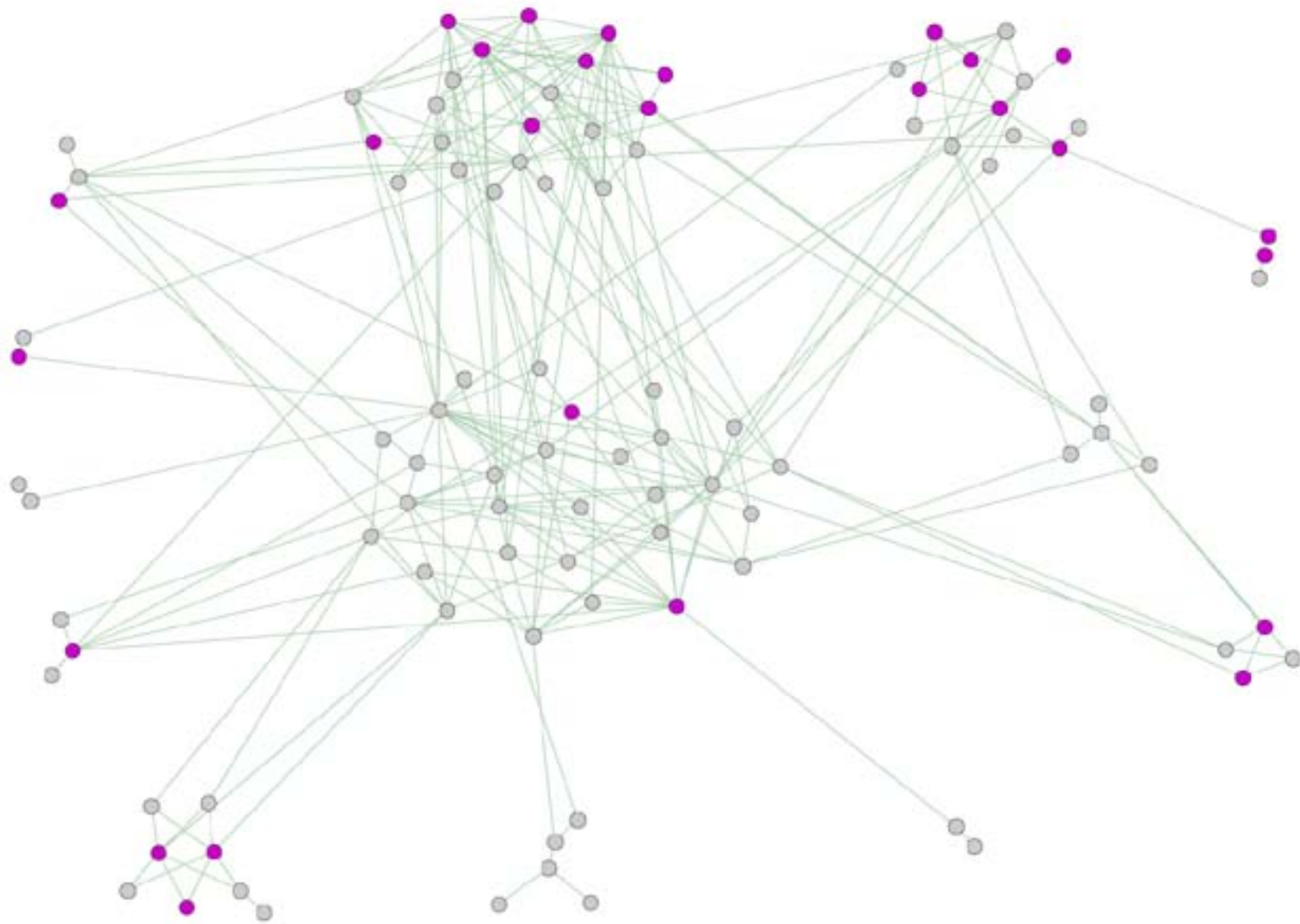


output pathways

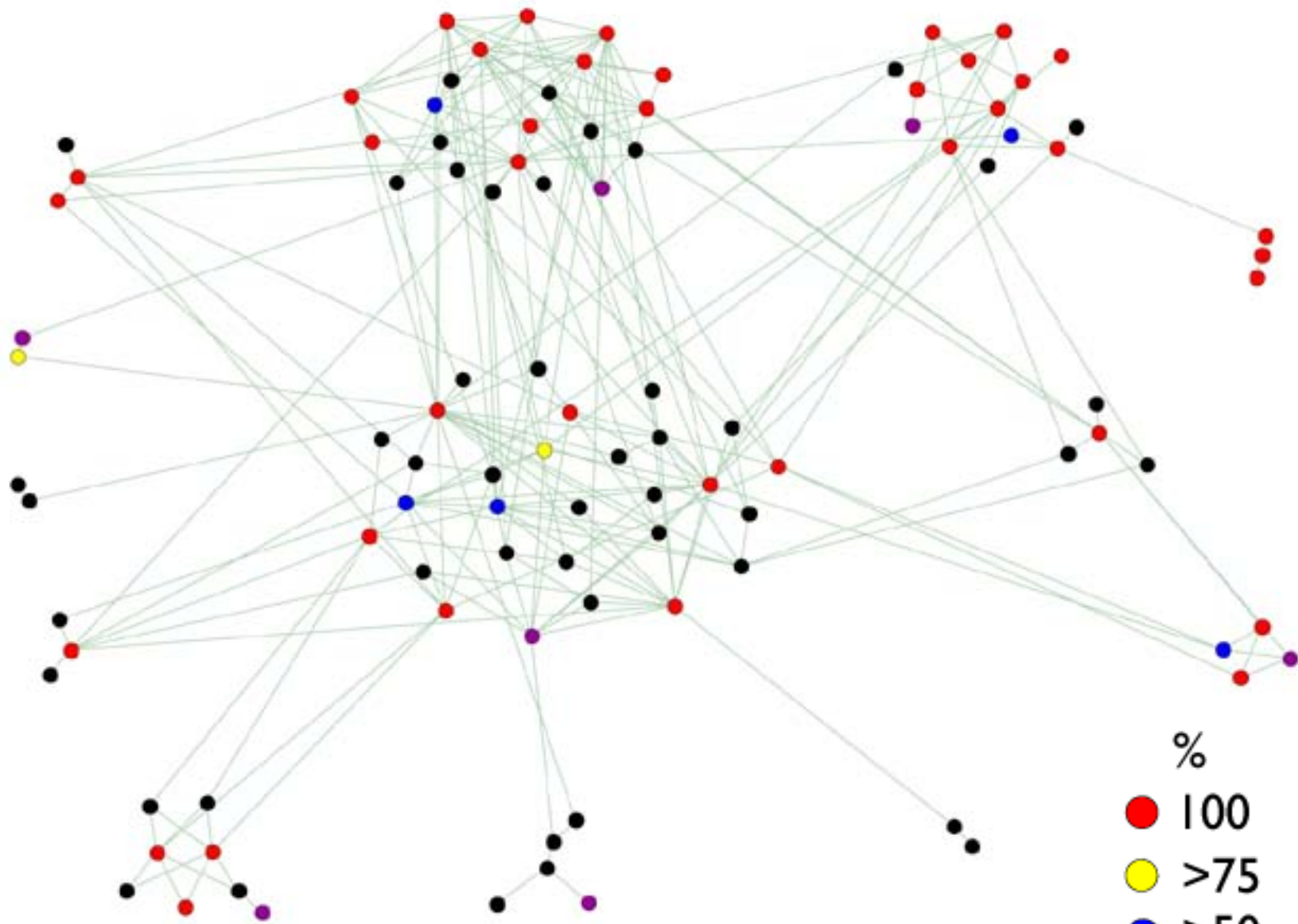


Evolutionary  
conservation  
is bottom up



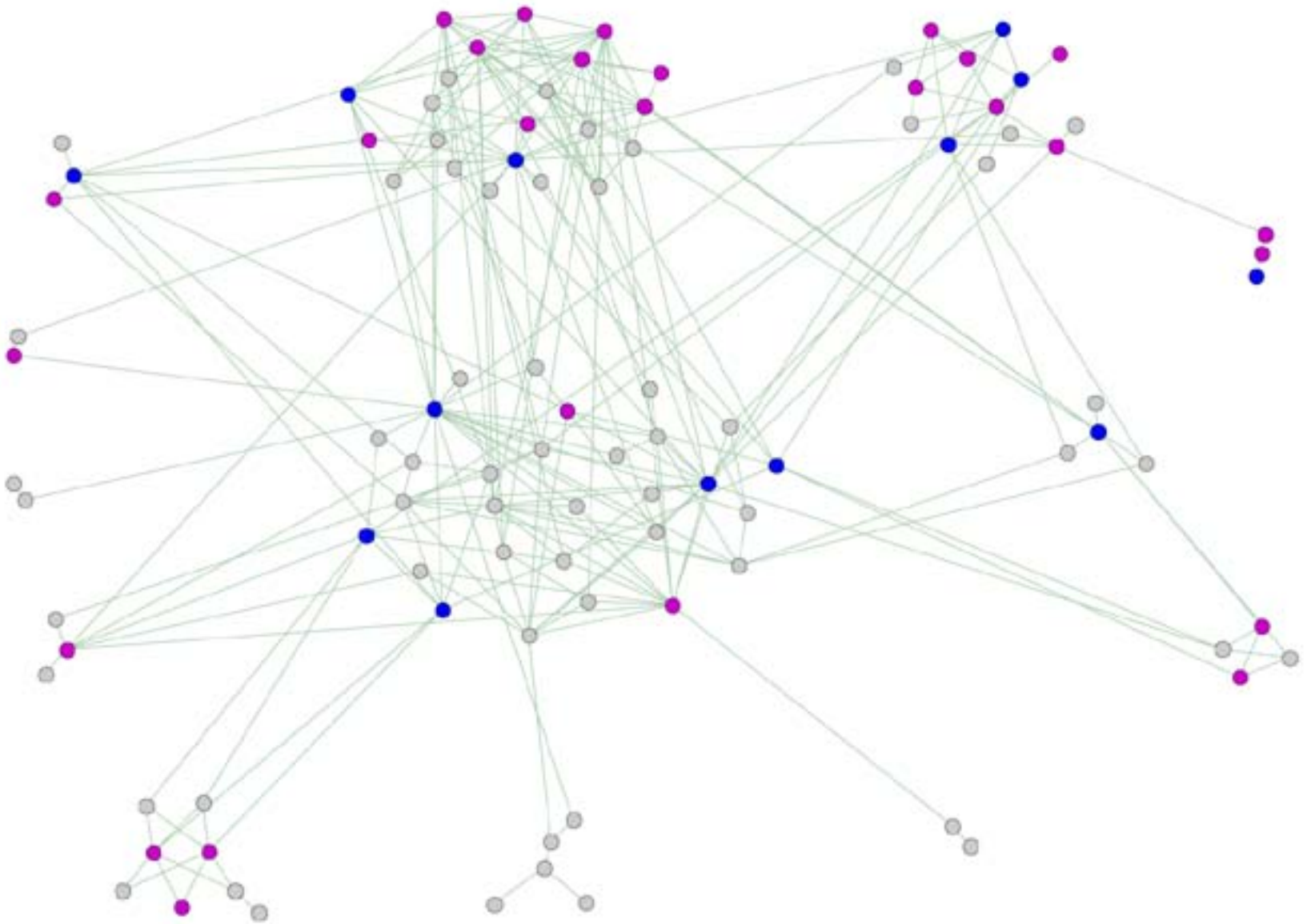


genes linked to schizophrenia (magenta)



% chance in predicted network

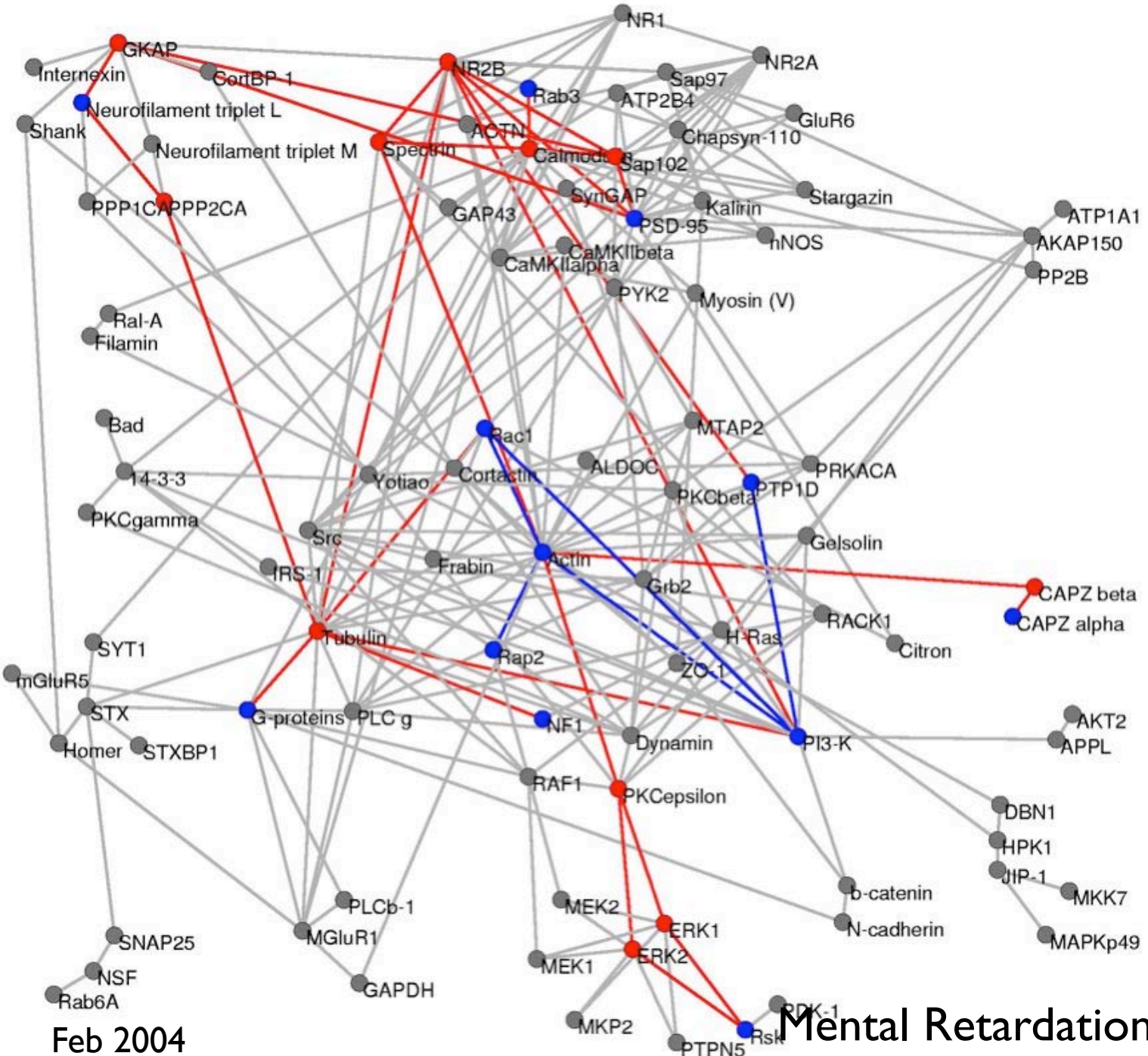
- %
- 100
  - >75
  - >50
  - >0
  - 0



testing by exon sequencing all nodes (> 1000 patients)

David Porteous, Douglas Blackwood, Walter Muir, Iain Deary





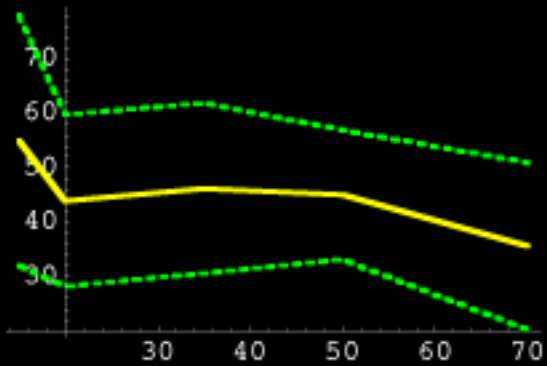
Feb 2004

Mental Retardation

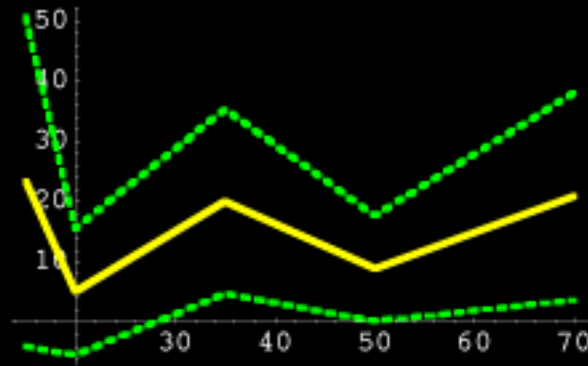
# robustness

% recovered : % data unused

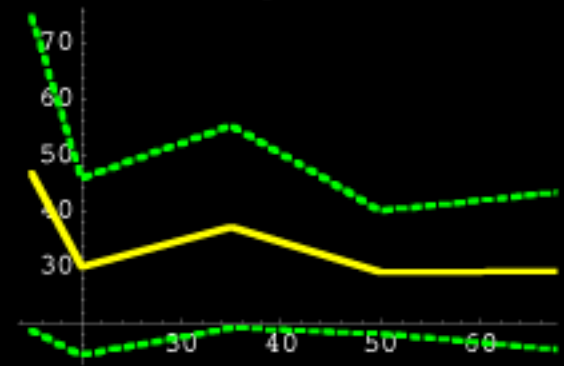
sch



ltp

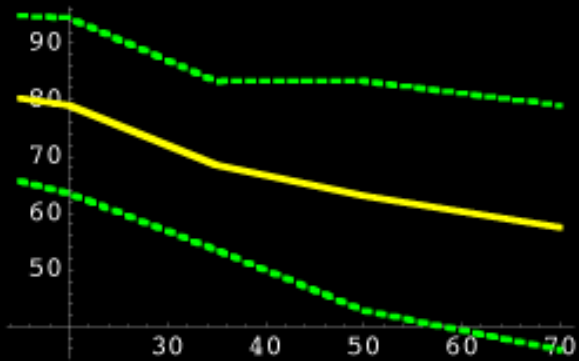


spatial

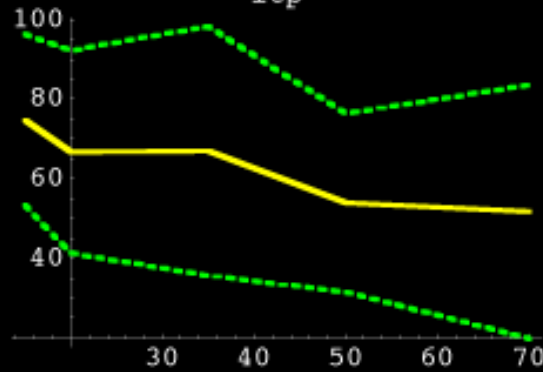


% overlap (predictions) : % data unused

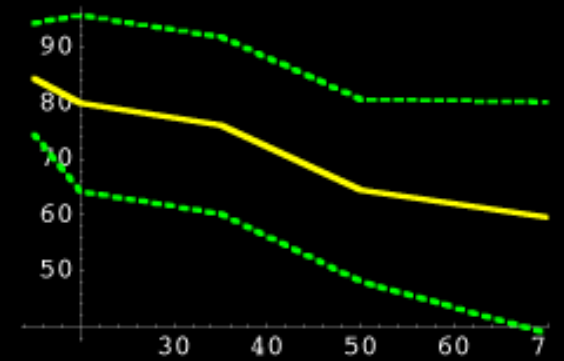
sch



ltp

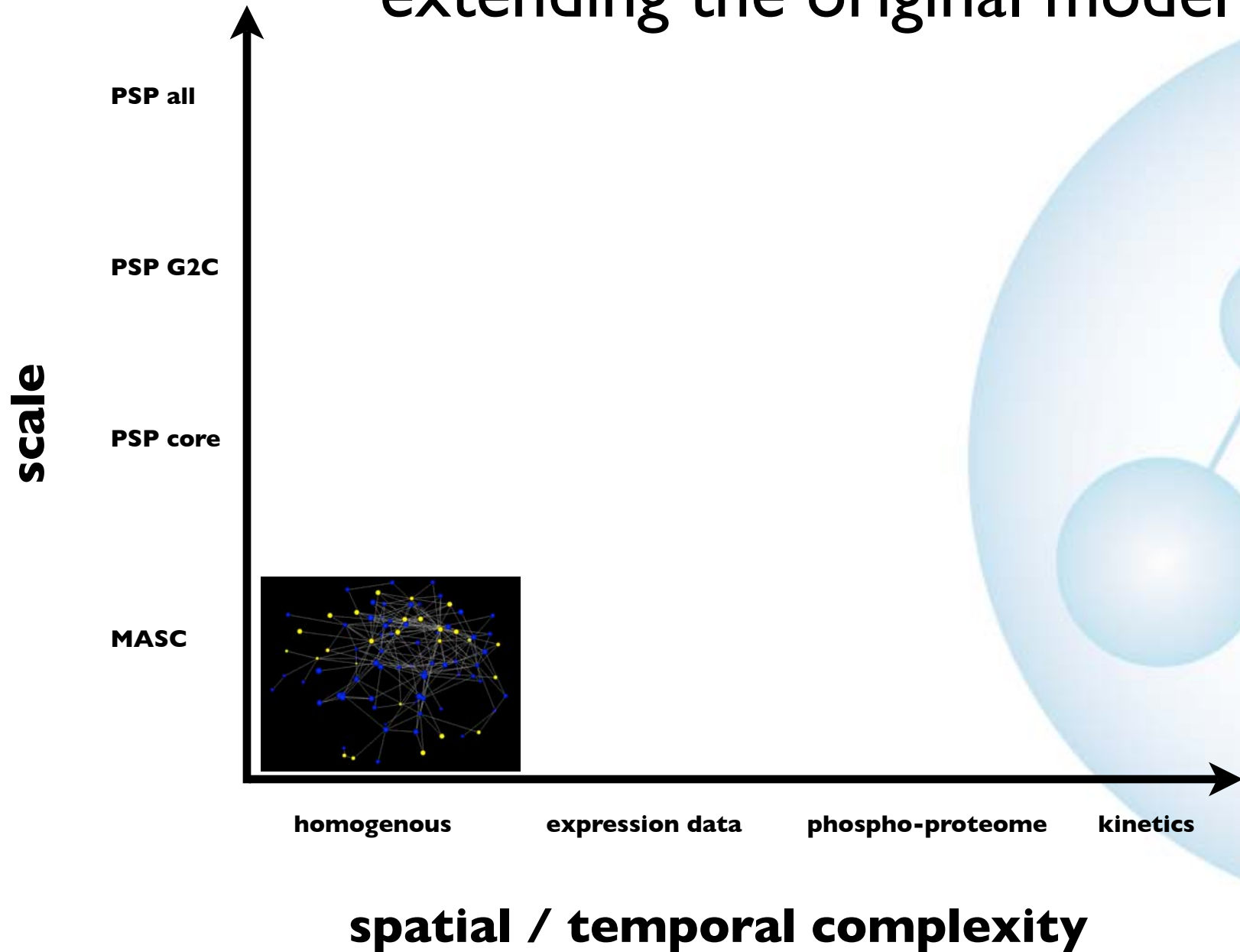


spatial

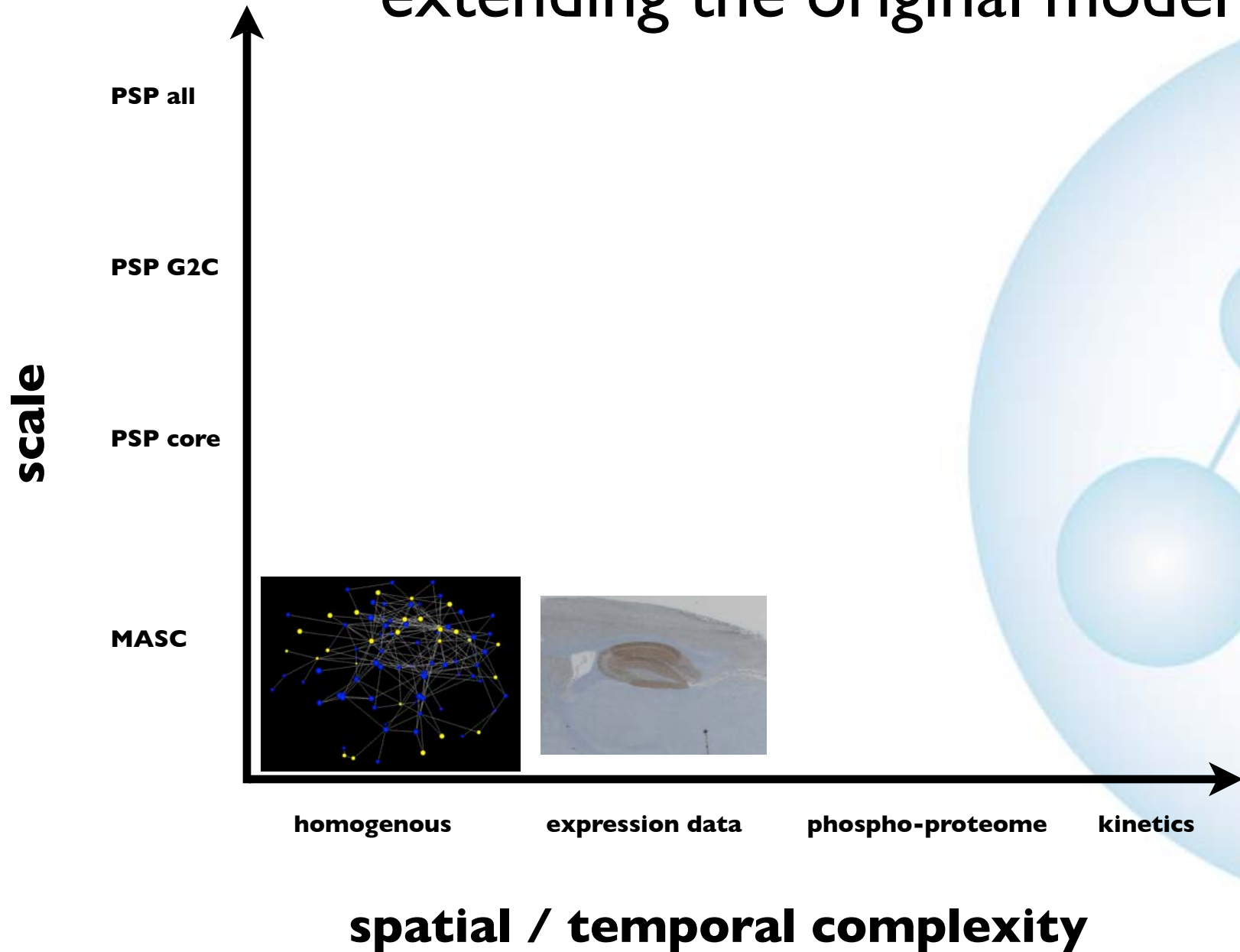


% original data removed

# extending the original model



# extending the original model



# extending the original model

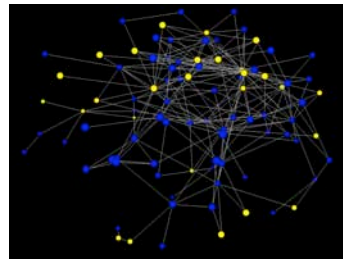
**scale**

PSP all

PSP G2C

PSP core

MASC



homogenous

expression data

phospho-proteome

kinetics

**spatial / temporal complexity**

# extending the original model

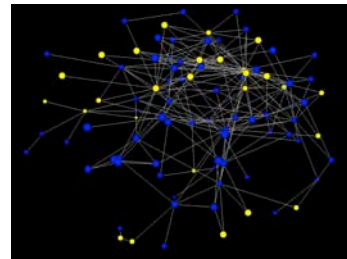
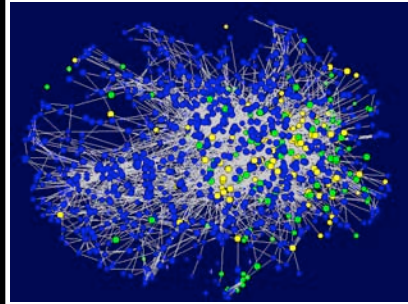
**scale**

PSP all

PSP G2C

PSP core

MASC



homogenous

expression data

phospho-proteome

kinetics

**spatial / temporal complexity**

# extending the original model

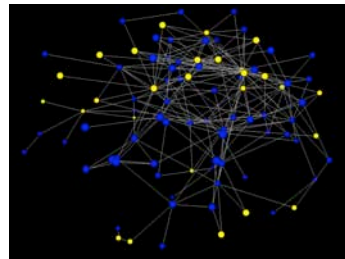
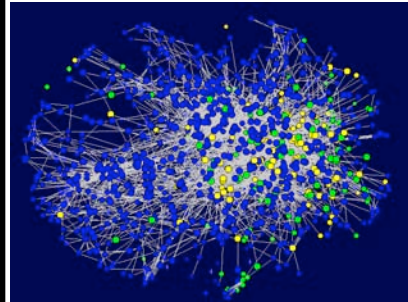
**scale**

PSP all

PSP G2C

PSP core

MASC



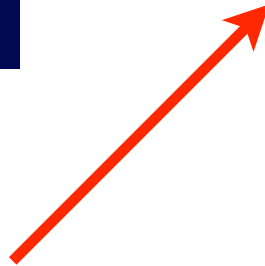
homogenous

expression data

phospho-proteome

kinetics

**spatial / temporal complexity**



# extending the original model

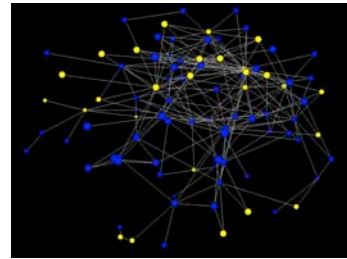
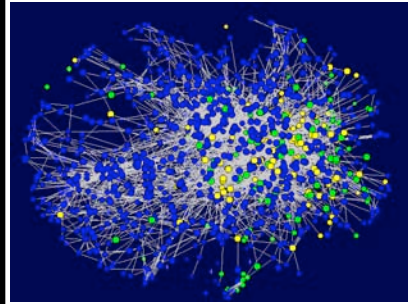
**scale**

PSP all

PSP G2C

PSP core

MASC



**first working model  
of synaptic function**

homogenous

expression data

phospho-proteome

kinetics

**spatial / temporal complexity**



# wet lab

# g2c

# informatics

Learning and memory  
Psychiatric Studies

Behaviour

Behaviour databases  
Computer vision tracking  
Machine learning behaviour

Volumetric analysis  
Connectivity mapping

Brain

LTP  
1° Neuron Culture  
*in vivo* calcium imaging  
Immunohistochemistry

Networks

Network models  
Gene expression databases  
LTP database

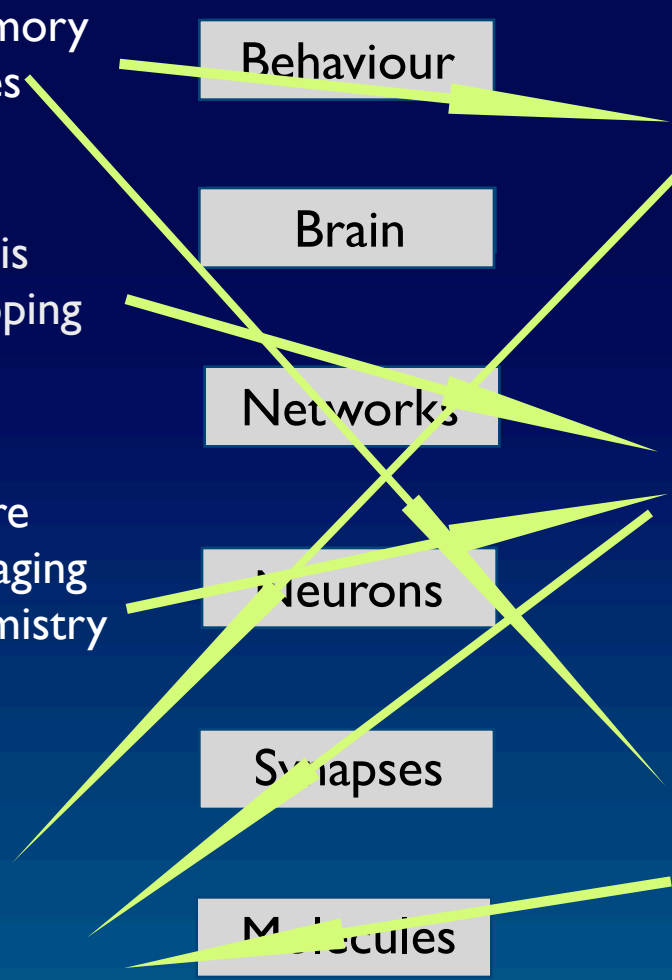
Proteomics

Synapses

Protein network modelling  
Mutational and SNP analysis  
Protein and Gene Databases

Gene Knock-outs  
Microarrays  
SNP mapping/sequencing

Molecules



[www.genes2cognition.org](http://www.genes2cognition.org)

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Mark Cumiskey - bio text mining & DBs

Holger Husi - protein interaction curation

Mike Marshall - curation

Richard Emes (UCL) - evolutionary analysis

Tom O'Dell(UCLA)- LTP experiments

Edinburgh