



# Genomics and Pathways Prediction and Dose-Response

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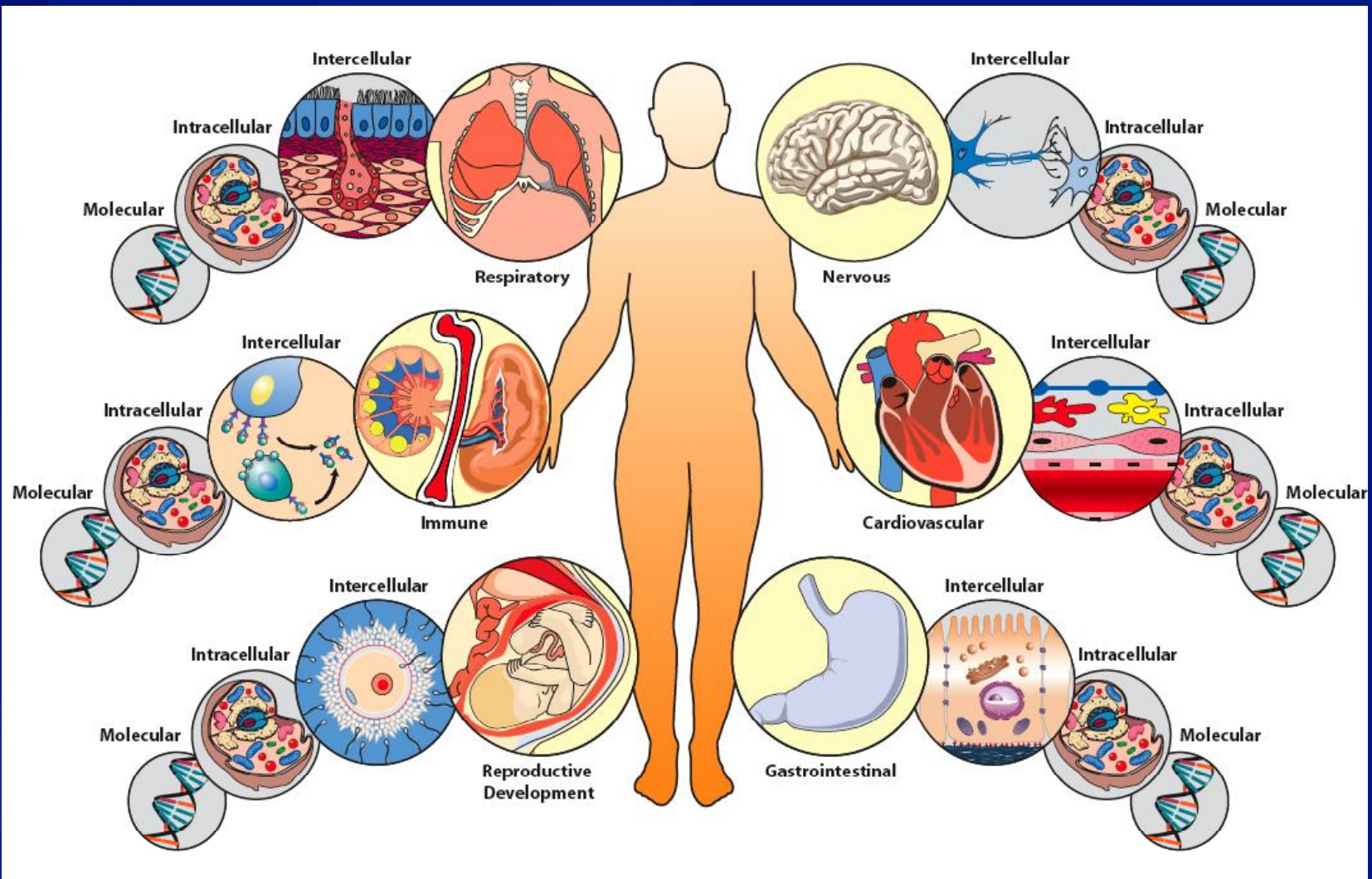
# Three Issues to Address

- q **Using pathway-based approaches**
  - § Strengthen findings seen in animals and humans
  - § Predict disease
- q **Cumulative risk assessment**
  - § Pathways as a common paradigm for linkage
- q **Mixtures**
  - § Pathways for identifying synergies
  - § We still need a method for addressing mixtures and risk

# Major Themes

- q **Measurement of multiplexed signals**
  - § Environmental
  - § Ecological
  - § Biological
- q **Greater acquisition and use of data from humans**
- q **Systems thinking (ecological frame of mind)**
- q **Complex analyses of complex systems**
  - § Ability to predict

# Systems Biology for the Individual



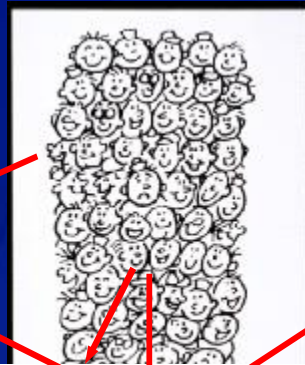
*Gohlke and Portier (2006)*

# Research and the Human Model

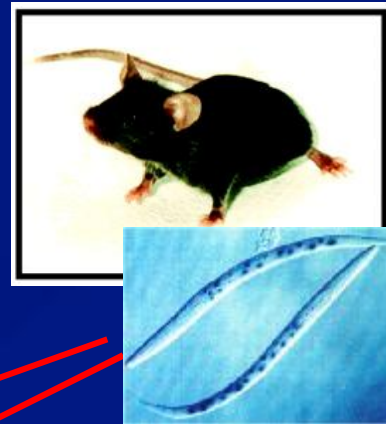
Human Clinical Laboratory



Epidemiology



Animal Models



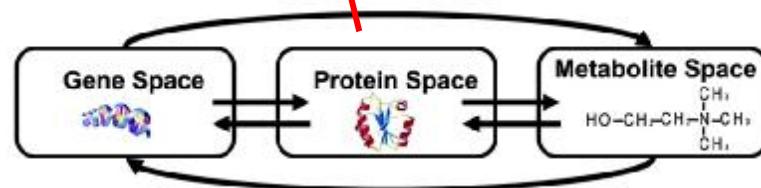
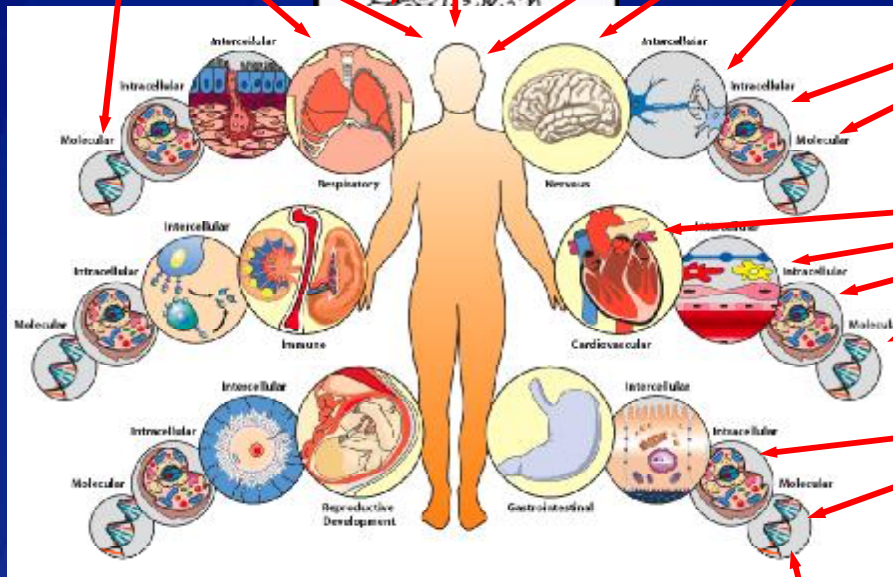
Tissue Cultures



Cell Cultures



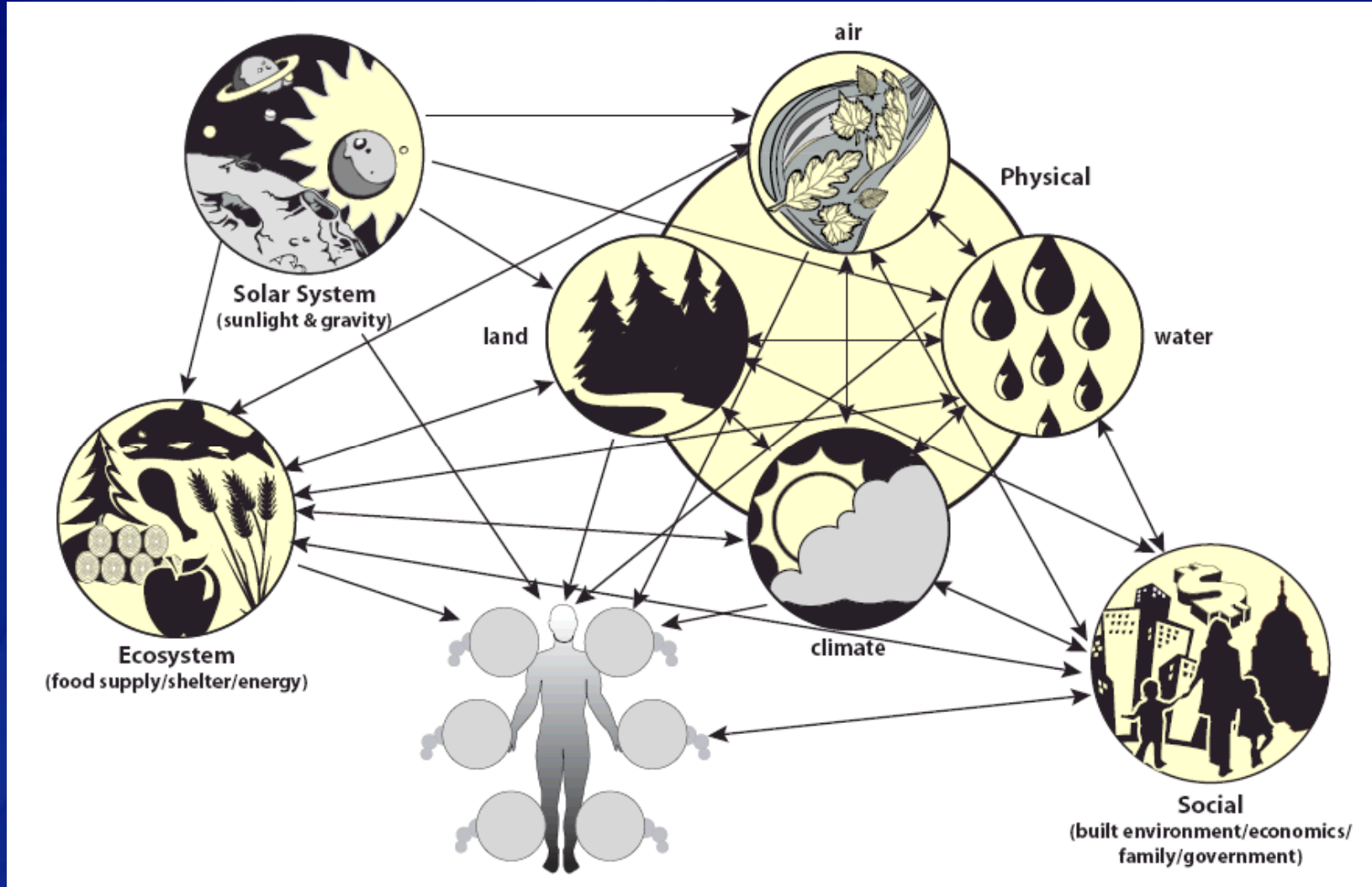
Molecular Biology



Gohlke and Portier (2006)

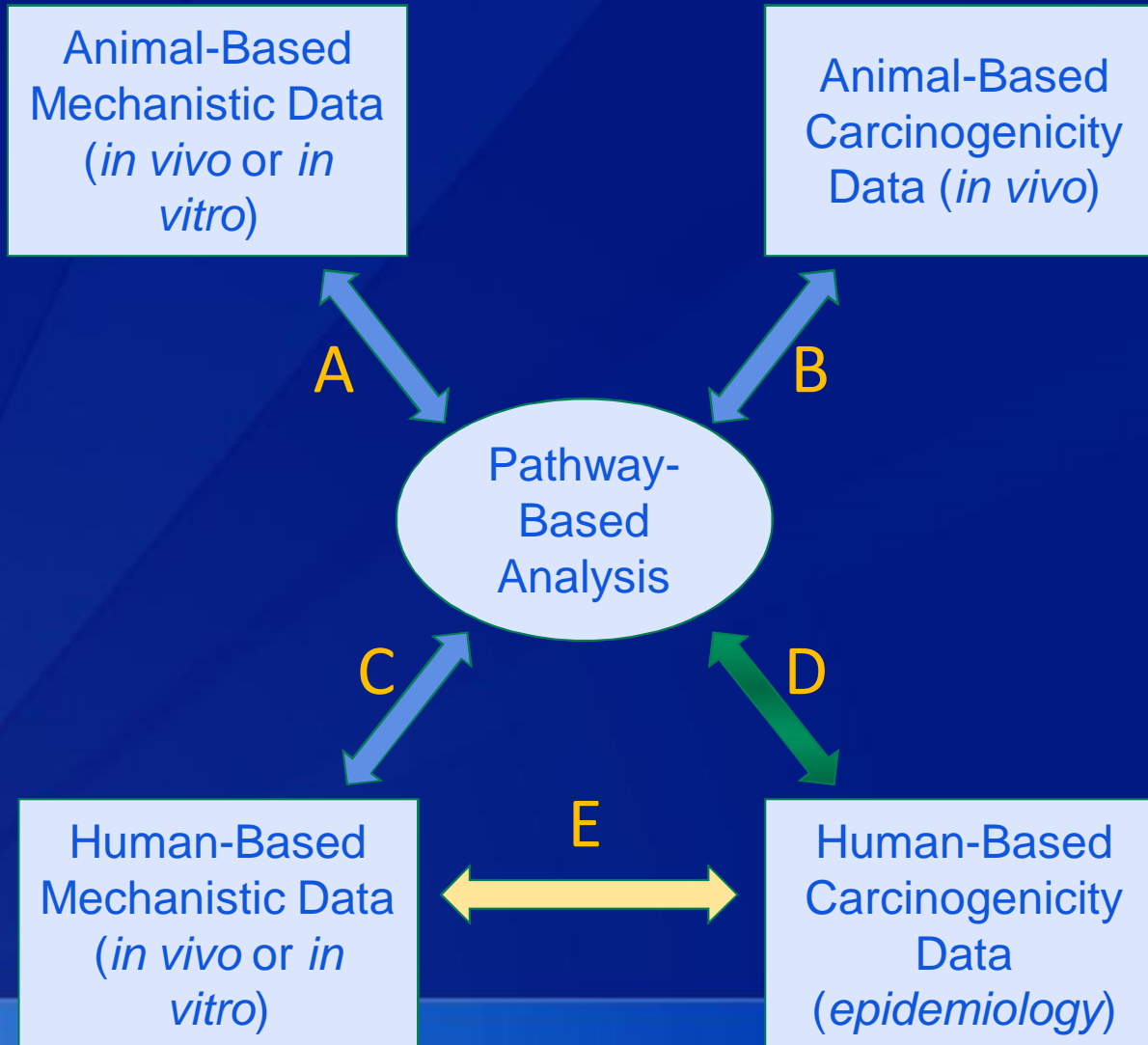
Boverhof and Zacharewski (Tox. Sci., 2006)

# Interaction Network: Our Environment and Our Health



*Gohlke and Portier (2007)*

# Predicting human health risk using pathways and their canonical structure



# Identifying Important Disease Pathways

Human Genetics  
+  
Human Disease

+

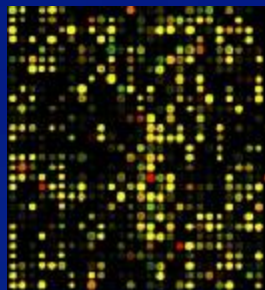


Sets of Pathways

Identify Pathways  
Related to Human  
Disease

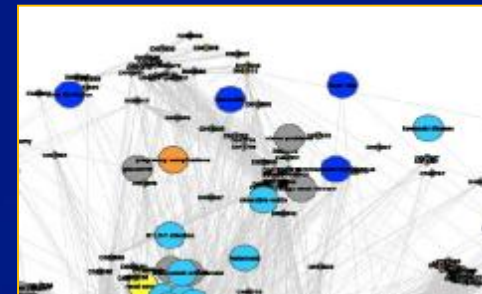


+



High Throughput  
High Content  
Chemical-Specific  
Data

Predict  
Chemical-Gen-  
Disease Interactome



Predict  
Risks

Develop  
Screens

Set  
Priorities

Fingerprint  
Toxicants

Develop  
Hypotheses

New 'omics  
& HTS  
Data

# Genetic Association Database

[geneticassociationdatabase.nih.gov](http://geneticassociationdatabase.nih.gov)

- The Genetic Association Database is a gene-centered archive of published scientific papers on human genetic association studies.
- Database Contents
  - 28347 records on human gene-phenotype (mostly complex disease) relationships
- Data used in our analysis
  - Manual phenotype grouping and better annotation
  - 8,825 unique associations between 2088 genes and 208 disease phenotypes

Becker et al. (2004) *Nature Genetics* 36(5)

# Comparative Toxicogenomics Database

<http://ctd.mdibl.org/>

- Interactions between environmental factors and genes/proteins in diverse organisms are curated from the published literature using both algorithm based methods as well as manual curation.
- Environmental factor identifiers used in the literature are annotated using MeSH chemical terms.

Mattingly et al. 2006. *Toxicol Sci.* 92(2):587-95.

# Unsupervised Clustering of Pathways with Phenotypes

Cardiovascular  
Metabolic

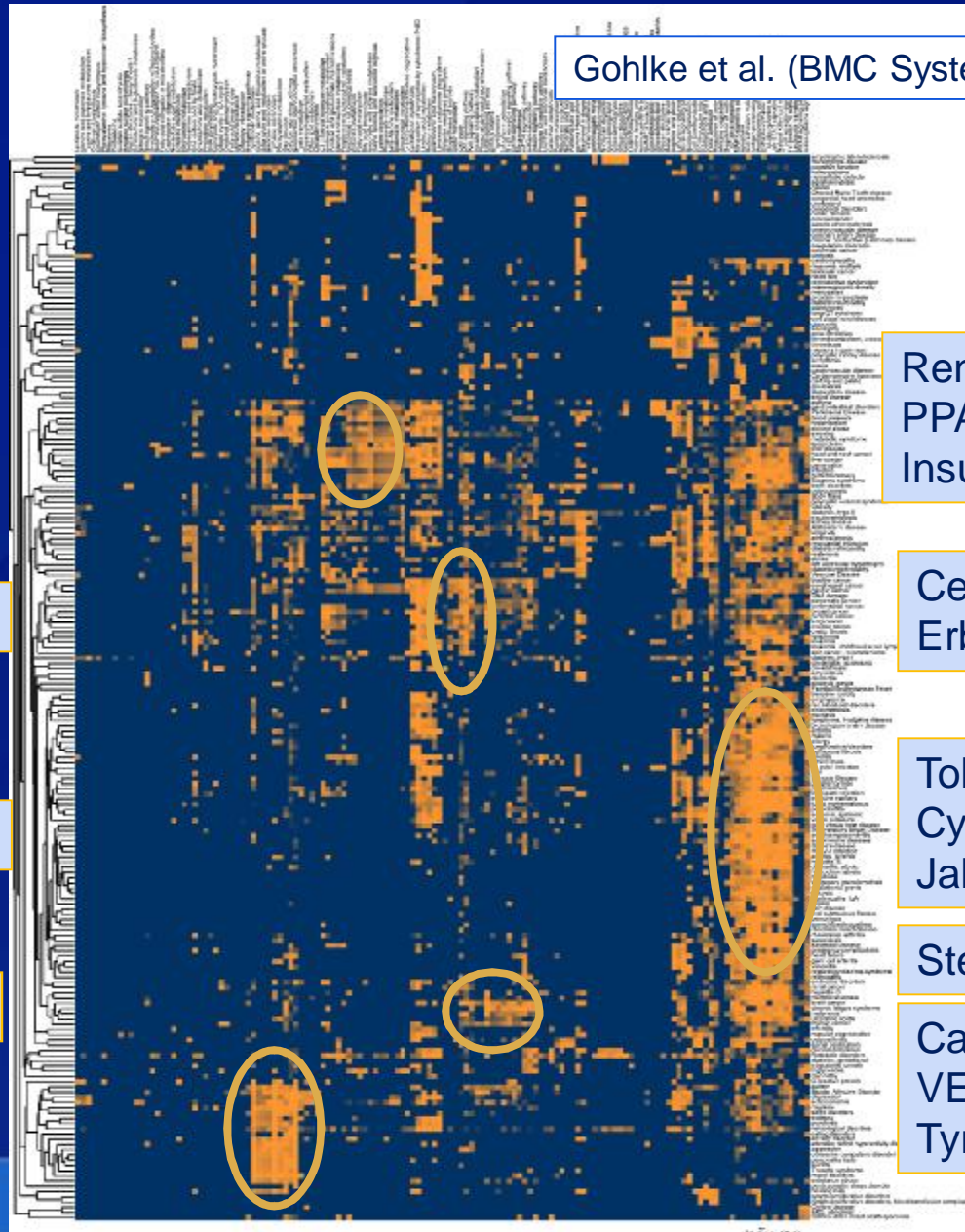
Cancer

Immune

Endocrine

Neuropsychiatric

Gohlke et al. (BMC Systems Biology, 2009)



Renin-angiotensin  
PPAR  
Insulin

Cell cycle  
ErbB

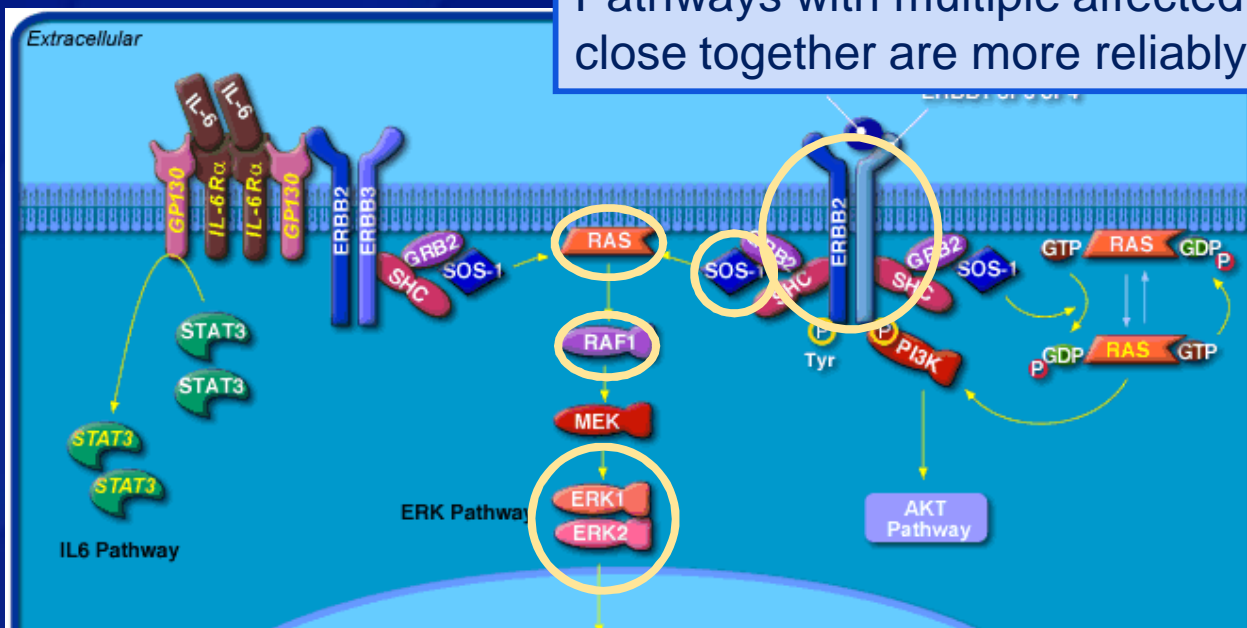
Toll like receptor  
Cytokine  
Jak-Stat

Steroid hormone

Calcium  
VEGF  
Tyrosine metabolism

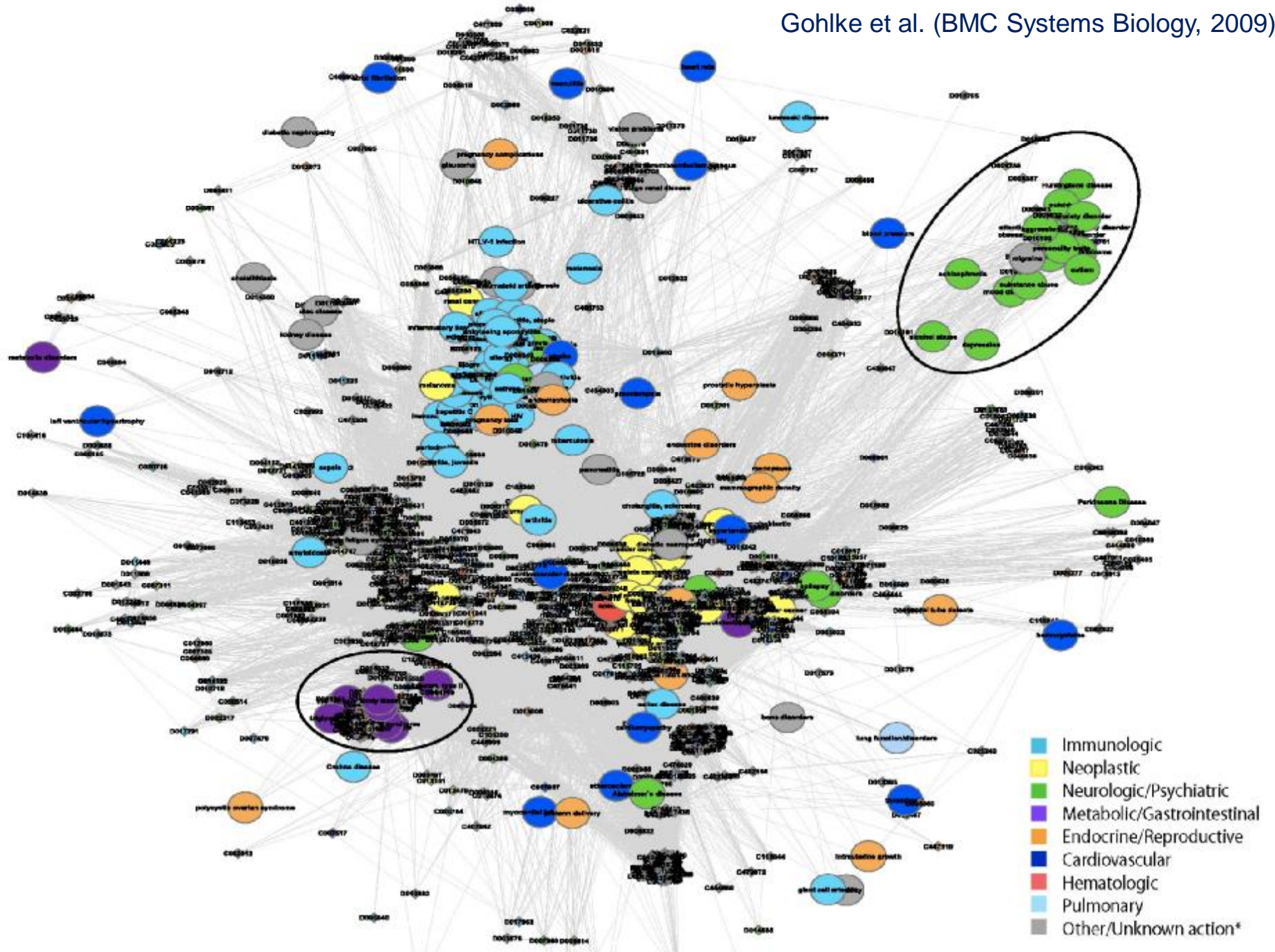
# Structurally-Enhanced Pathway Enrichment

Sequential Best Rule  
Pathways with multiple affected components close together are more reliably linked

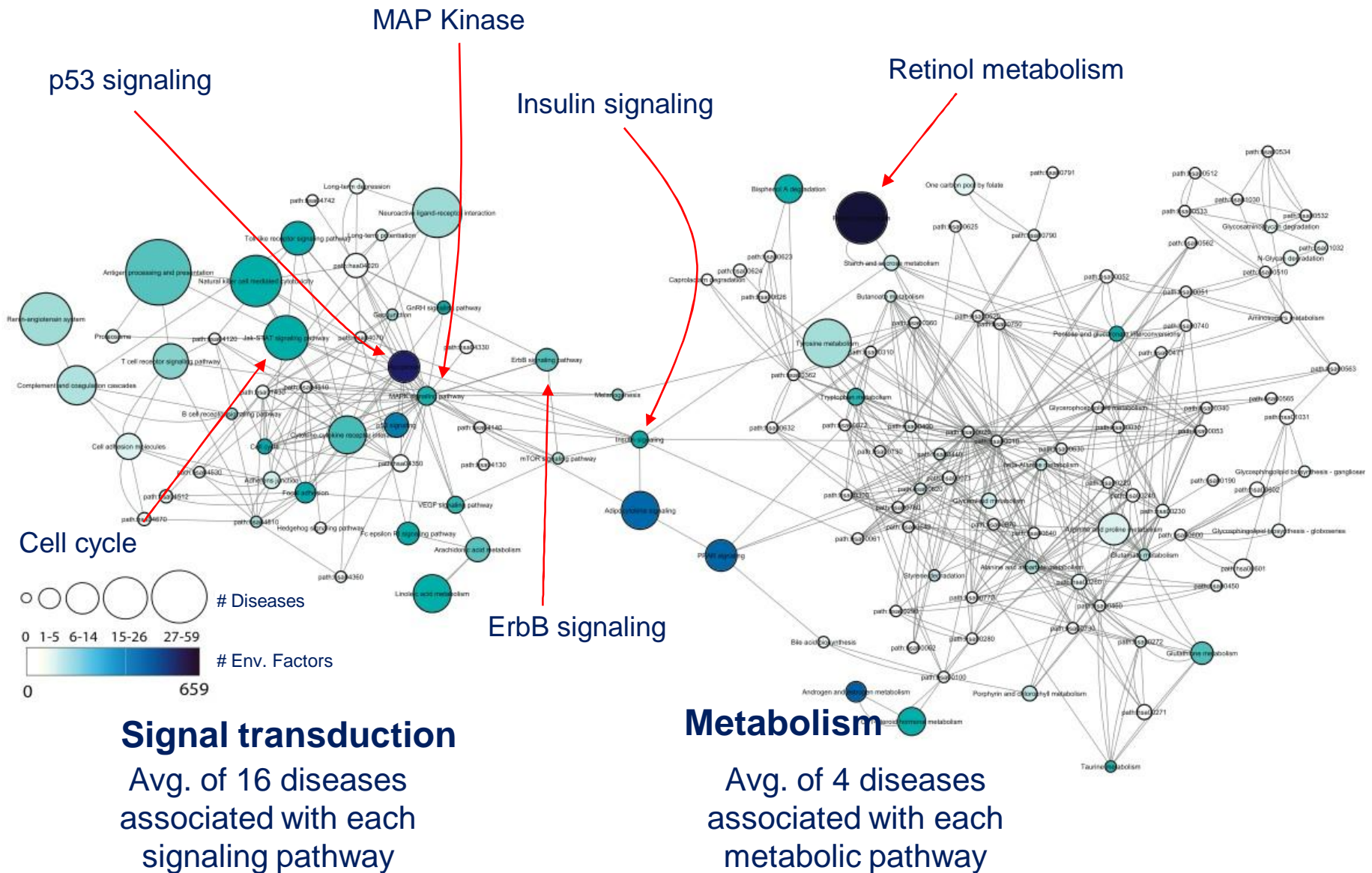


Heavy Ends Rule  
Specificity of the pathway at the receptor end  
Ultimate response by downstream components  
Intermediate components typically shared between pathways





# Relating Across Pathways



# Critical Toxicity Pathways:

## q Ranked highly for both phenotype and environmental factor

§ Metabolism by cytochrome P450

§ Retinol metabolism

§ Jak-STAT signaling

§ Adipocytokine signaling

§ Toll-like receptor signaling

## q Other important pathways

§ Apoptosis

§ p53 signaling pathway

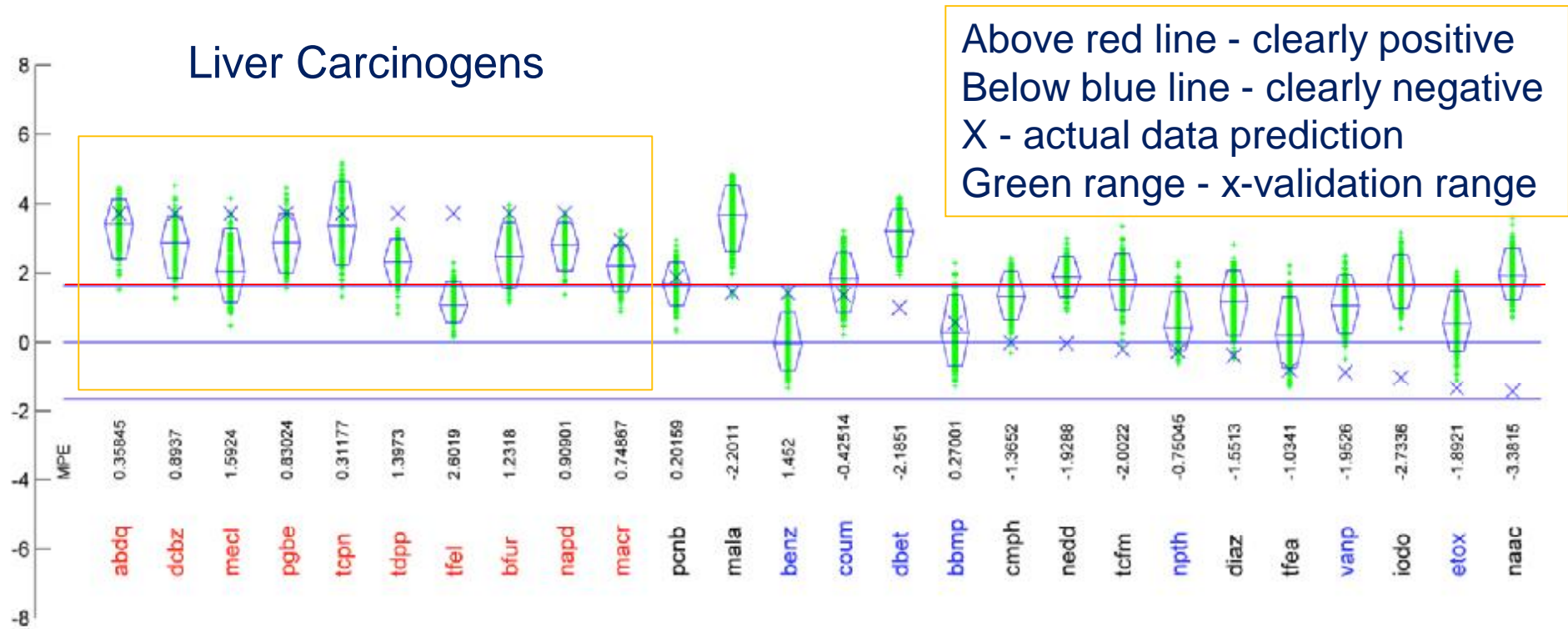
§ Cell-cycle pathway

§ PPAR signaling pathway

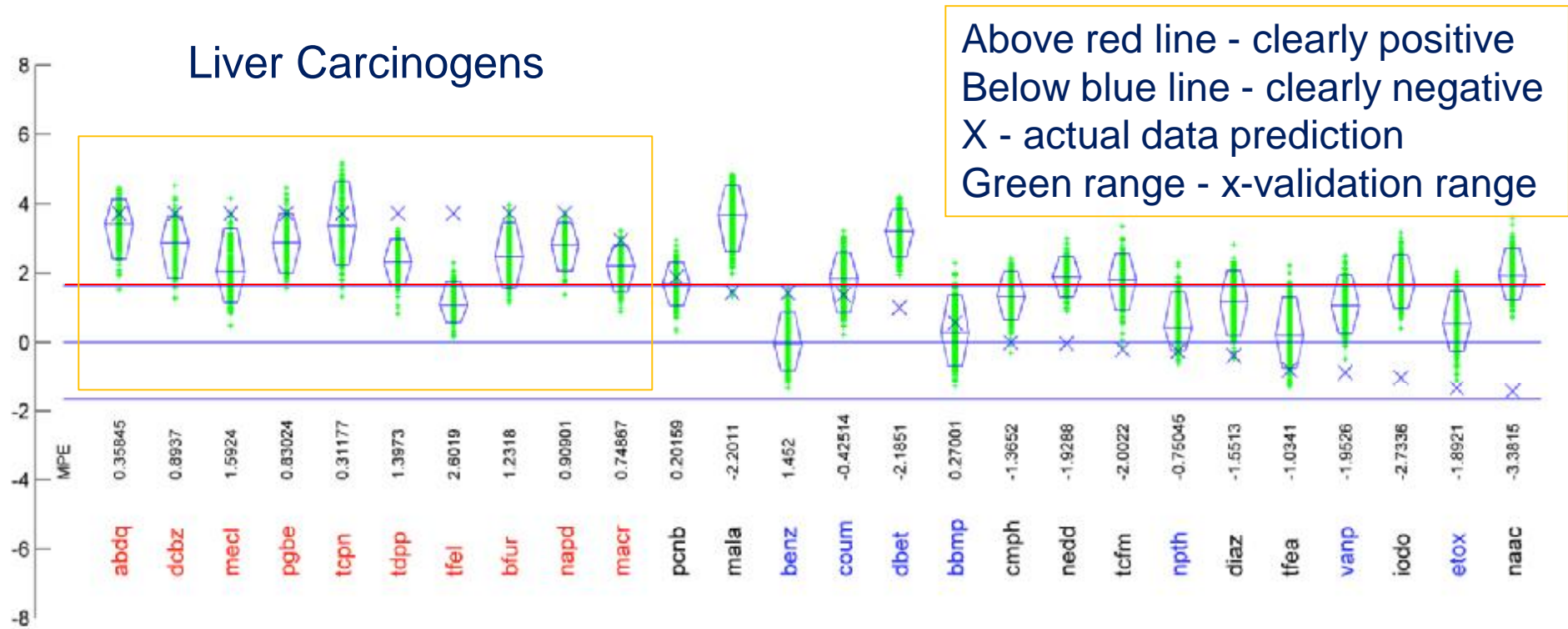
# Microarray data from B6C3F<sub>1</sub> mice following 90 day exposure

Chemical	Short Name	NTP No.	Route <sup>a</sup>	Dose	Tumors
1-Amino-2,4-dibromoanthraquinone	ADBQ	383	Food	20,000 ppm	Liver
Methylene Chloride	MECL	306	Inhalation	4,000 ppm	Liver
N-Methylolacrylamide	MACR	352	Gavage (Water)	50 mg/kg	Liver
Tris(2,3-dibromopropyl)phosphate	TDPP	76	Food	1,000 ppm	Liver
2,2-Bis(bromomethyl)-1,3-propanediol	BBMP	452	Food	1,250 ppm	Other
1,2-Dibromoethane	DBET	86	Gavage (CO)	62 mg/kg	Other
Ethylene Oxide	ETOX	326	Inhalation	100 ppm	Other
Naphthalene	NPTH	410	Inhalation	30 ppm	Other
Vanadium Pentoxide	VANP	507	Inhalation	2.0 mg/m <sup>3</sup>	Other
1,4-Dichlorobenzene	DCBZ	319	Gavage (CO)	600 mg/kg	Liver
Propylene glycol mono- <i>t</i> -butyl ether	PGBE	515	Inhalation	1,200 ppm	Liver
Tetrafluoroethylene	TFEL	450	Inhalation	1,250 ppm	Liver
1,2,3-Trichloropropane	TCPN	384	Gavage (CO)	60 mg/kg	Liver
2-Chloromethylpyridine hydrochloride	CMPH	178	Gavage (Water)	250 mg/kg	No
Diazinon	DIAZ	137	Food	200 ppm	No
Iodoform	IODO	110	Gavage (CO)	93 mg/kg	No
Malathion	MALA	24	Food	14,800 ppm	No
4-Nitroanthranilic acid	NAAC	109	Food	10,000 ppm	No
Tetrafluoroethane	TFEA	--- <sup>d</sup>	Inhalation	50,000 ppm	No
Trichlorofluoromethane	TCFM	106	Gavage (CO)	3,925 mg/kg	No
Air	ACON		Inhalation		
Corn oil	CCON		Gavage (CO)		
Feed	FCON		Food		
Water	WCON		Gavage (Water)		

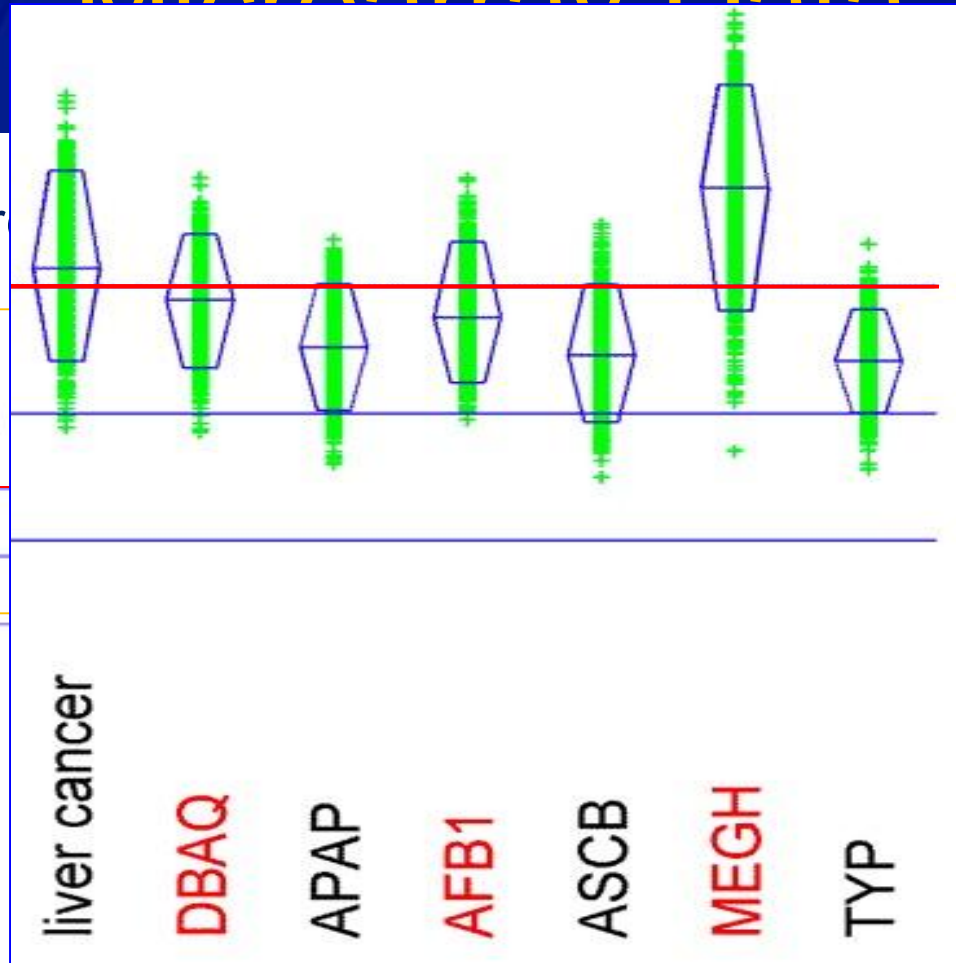
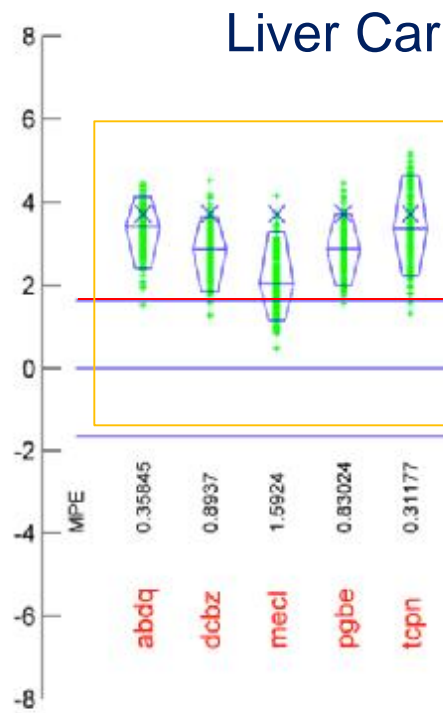
# Predicted Liver Cancer from Microarray Data



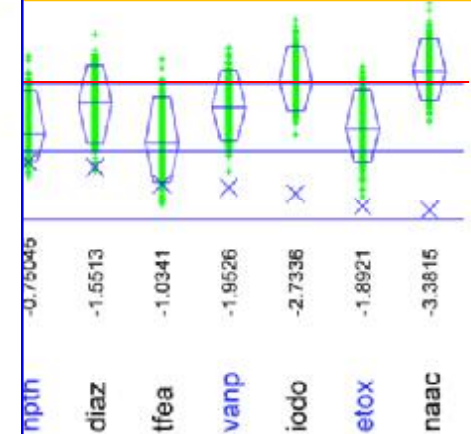
# Predicted Liver Cancer from Microarray Data

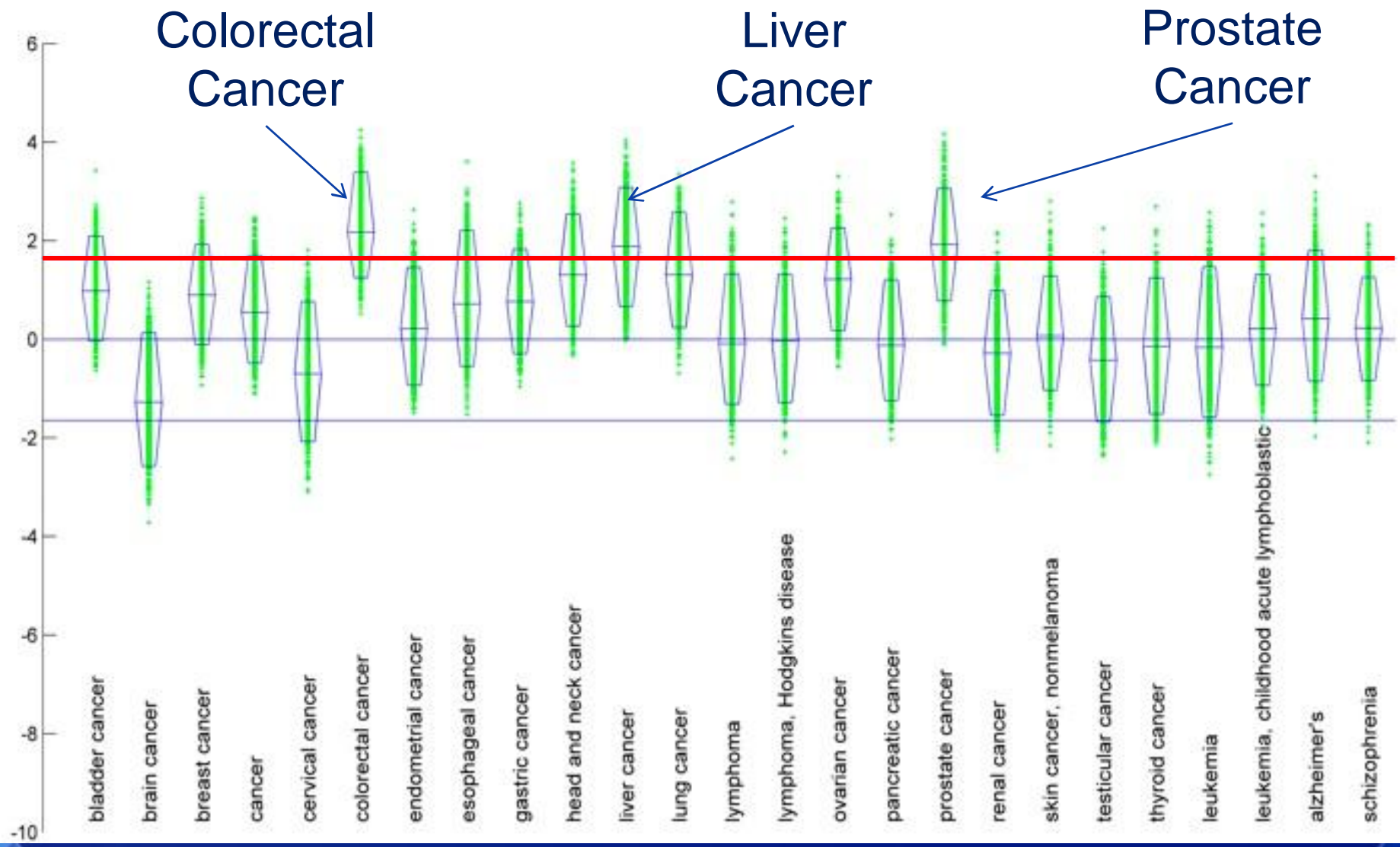


# Predicted Liver Cancer from Microarray Data



red line - clearly positive  
 blue line - clearly negative  
 data prediction  
 yellow box - x-validation range





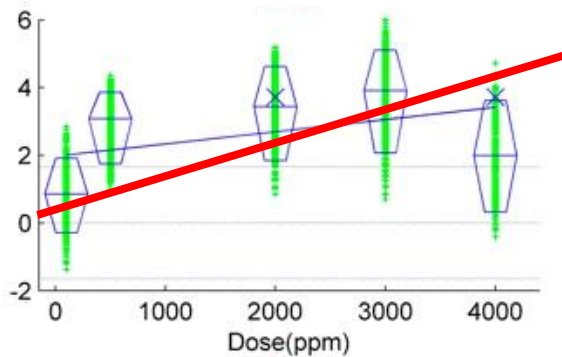
Colorectal  
Cancer

Liver  
Cancer

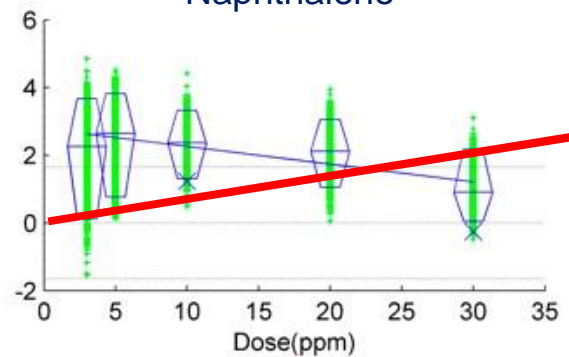
Prostate  
Cancer

# Evaluating Dose-Response Patterns

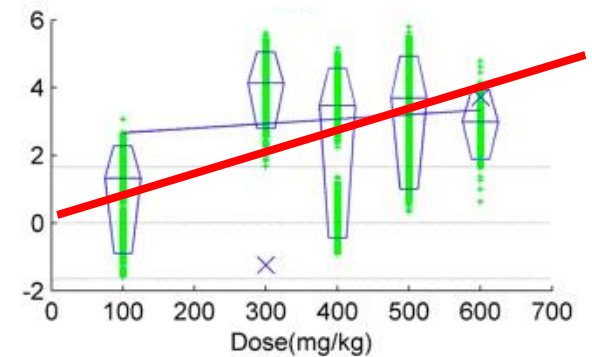
Methylene Chloride



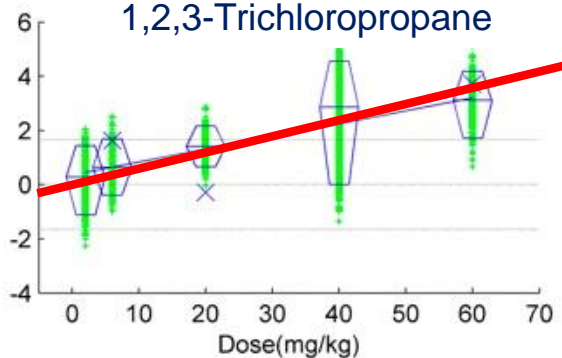
Naphthalene



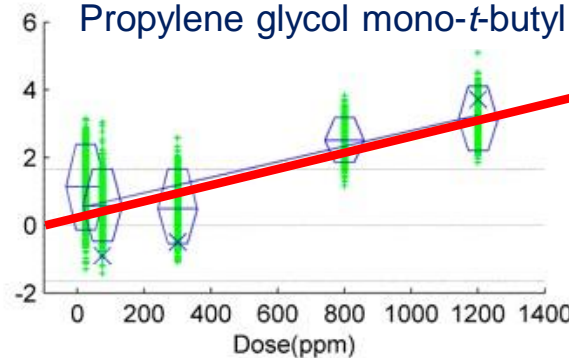
1,4-Dichlorobenzene



1,2,3-Trichloropropane



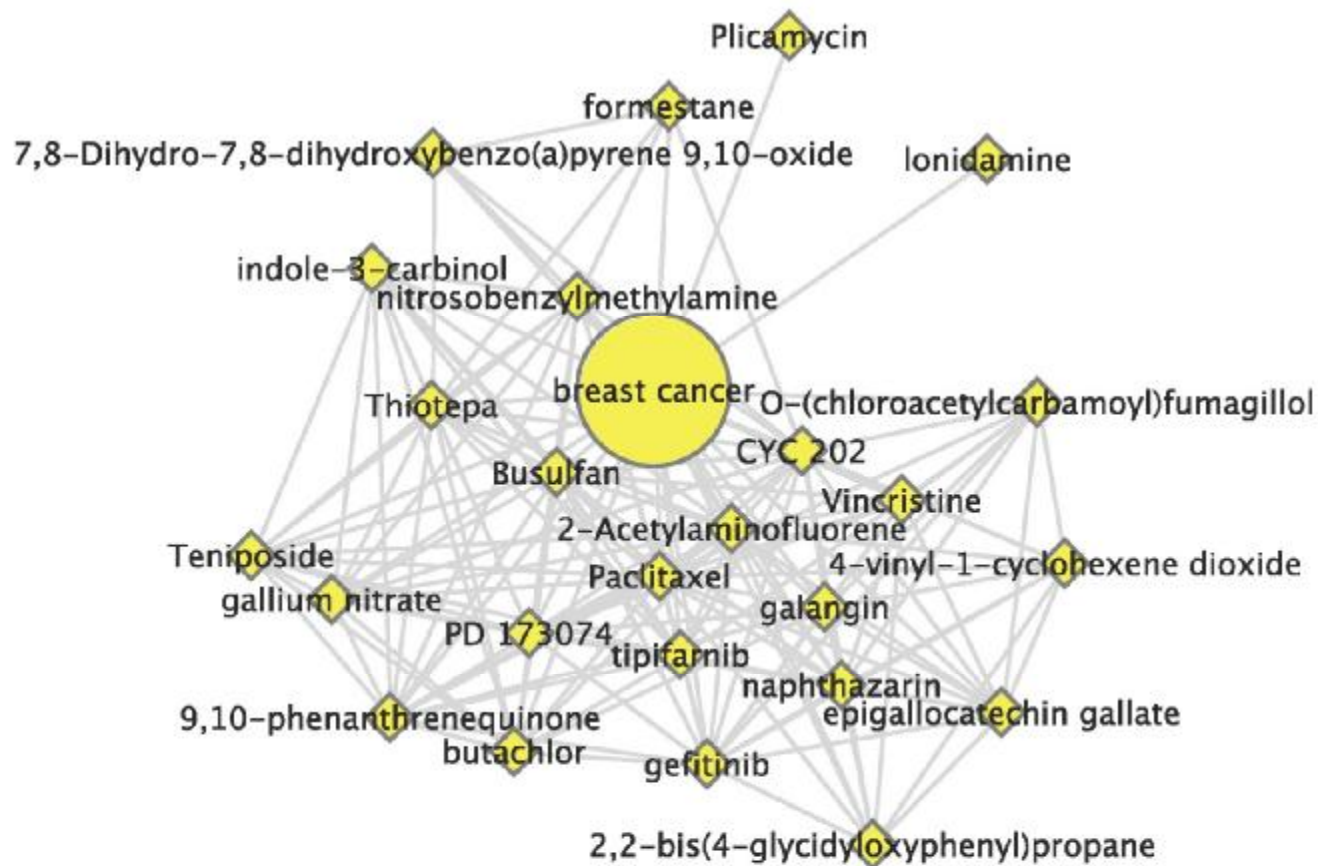
Propylene glycol mono-*t*-butyl ether



# Cumulative Risk Assessment

- q Pathways can be used to identify compounds with common activation patterns
- q These can be linked with specific diseases
- q Dose additivity can be used if pathways seem to be altered in similar directions
  - § Up- and down-regulations are identical
- q More complicated if patterns of alteration are different

# Breast Cancer & Environment Interactome



## Mixtures with Different Mechanisms

- q Pathways can help to distinguish mechanisms
- q Need a new paradigm for aggregating risks
  - § Dose additivity won't work
  - § Aggregation needs to be done in the risk range

# Acknowledgements

- Environmental Systems Biology
  - **Julia Gohlke**
  - **Reuben Thomas**
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  - Xiaoqing Chang
  - Melissa Chan
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  - Nigel Walker
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- Mount Desert Island Biological Laboratory
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- Hamner Institute
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