United States Environmental Protection Agency

Profiling 976 ToxCast Chemicals across 331 Enzymatic and Receptor Signaling Assays

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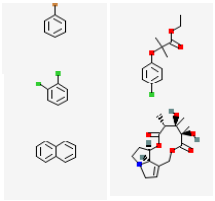
EPA's Computational Toxicology Communities of Practice
US EPA, May 23, 2012

Office of Research and Development

ToxCast™ Program

Chemical prioritization using in vitro to in vivo correlations

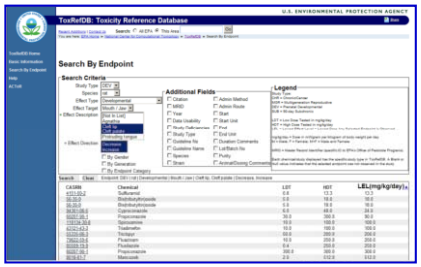
Chemical library



1000s chemicals

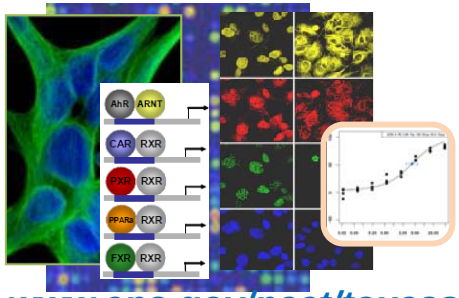
- food use pesticides, failed pharmaceuticals, plasticizers, and food additives

in vivo data



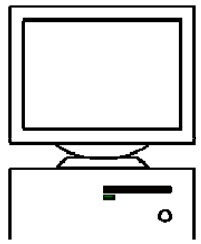
www.epa.gov/ncct/toxrefdb/

in vitro testing



www.epa.gov/ncct/toxcast/

NovaScreen Biochemical Platform



Bioinformatics

More chemicals screened

Less time and cost

Fewer animals

Based on in vitro targets, pathways and processes

Chemical Research in Toxicology

Article

pubs.acs.org/crt

Profiling 976 ToxCast Chemicals across 331 Enzymatic and Receptor Signaling Assays

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Supporting Information

ABSTRACT: Understanding potential health risks is a significant challenge due to the large numbers of diverse chemicals with poorly characterized exposures and mechanisms of toxicities. The present study analyzes 976 chemicals (including failed pharmaceuticals, alternative plasticizers, food additives, and pesticides) in Phases I and II of the U.S. EPA's ToxCast project across 331 cell-free enzymatic and ligand-binding high-throughput screening (HTS) assays. Half-maximal activity concentrations (AC50) were identified for 729 chemicals in 256 assays (7,135 chemical–assay pairs). Some of the most commonly affected assays were CYPs (CYP2C9 and CYP2C19), transporters (mitochondrial TSPO, norepinephrine, and dopaminergic), and GPCRs (aminergic). Heavy metals, surfactants, and dithiocarbamate fungicides showed promiscuous but distinctly different patterns of activity, whereas many of the pharmaceutical compounds showed promiscuous activity across GPCRs. Literature analysis confirmed >50% of the activities for the most potent chemical–assay pairs (54) but also revealed 10 missed interactions. Twenty-two chemicals with known estrogenic activity were correctly identified for the majority (77%), missing only the weaker interactions. In many cases, novel findings for previously unreported chemical–target combinations clustered with known chemical–target interactions. Results from this large inventory of chemical–biological interactions can inform read-across methods as well as link potential targets to molecular initiating events in adverse outcome pathways for diverse toxicities.

Phase I & II, NS Sipes et al 2013, *Chemical Research in Toxicology* **Article ASAP, DOI:** 10.1021/tx400021f

Phase I, TB Knudsen et al 2011, *Toxicology* **282(1-2):1-15, DOI:** 10.1016/j.tox.2010.12.01

NovaScreen Biochemical Assays

-Caliper Life Sciences (a PerkinElmer company)

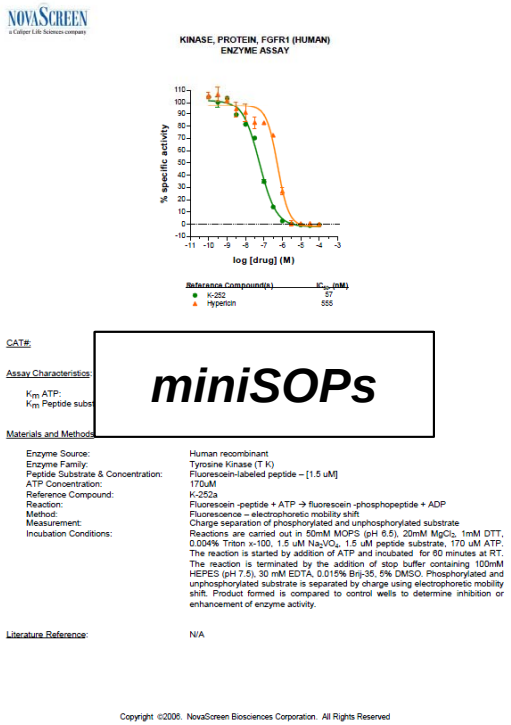
Purified or recombinant protein

Species

- Human (73%)
- Rat (18%)
- Other (9%)

Binding and enzymatic activity

- Radioligand receptor binding
- Fluorescent receptor binding
- Fluorescent enzyme substrate - intensity quench
- Fluorescent enzyme substrate - mobility shift


NOVASCREEN

KINASE, PROTEIN, FGFR1 (HUMAN)
ENZYME ASSAY

% specific activity

log [drug] (M)

Reference Compound(s)	IC_{50} (nM)
K-252	57
Hypericin	555

CAT#:

Assay Characteristics:

K_m ATP:
K_m Peptide subs

Materials and Methods:

Enzyme Source:	Human recombinant
Enzyme Family:	Tyrosine Kinase (T K)
Peptide Substrate & Concentration:	Fluorescein-labeled peptide – [1.5 uM]
ATP Concentration:	170uM
Reference Compound:	K-252a
Reaction:	Fluorescein -peptide + ATP → fluorescein -phosphopeptide + ADP
Method:	Fluorescence – electrophoretic mobility shift
Measurement:	Charge separation of phosphorylated and unphosphorylated substrate
Incubation Conditions:	Reactions are carried out in 50mM MOPS (pH 6.5), 20mM $MgCl_2$, 1mM DTT, 0.004% Triton x-100, 1.5 uM Na_2VO_4, 1.5 uM peptide substrate, 170 uM ATP. The reaction is started by addition of ATP and incubated for 60 minutes at RT. The reaction is terminated by the addition of stop buffer containing 100mM HEPES (pH 7.5), 30 mM EDTA, 0.015% Brij-35, 5% DMSO. Phosphorylated and unphosphorylated substrate is separated by charge using electrophoretic mobility shift. Product formed is compared to control wells to determine inhibition or enhancement of enzyme activity.

Literature Reference: N/A

miniSOPs

http://www.perkinelmer.com/services/contractresearch/default.xhtml

NovaScreen Biochemical Assays

-Caliper Life Sciences (a PerkinElmer company)

976 chemicals in 331 biochemical assays

	Protein families	# assays
Binding	G-protein-coupled-receptors	77
	Ion channels	20
	Nuclear receptors	19
	Transporters	11
	Other receptors	3
Enzymatic activity Inhibition & Activation	Kinases	74
	Phosphatases	38
	Proteases	30
	Cytochrome P450 enzymes	20
	Cholinesterase	6
	Other enzyme (COX, HDAC, MAO, PDE, SIRT)	33

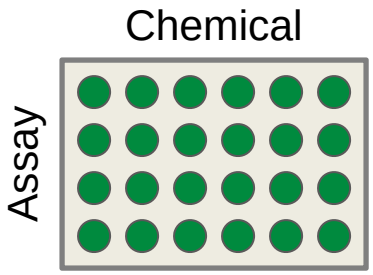
Sipes et al 2013, CRT

NovaScreen Workflow

-Caliper Life Sciences (a PerkinElmer company)

Chemical selection
Experimental design
Ordering
Assay annotation

Step 1

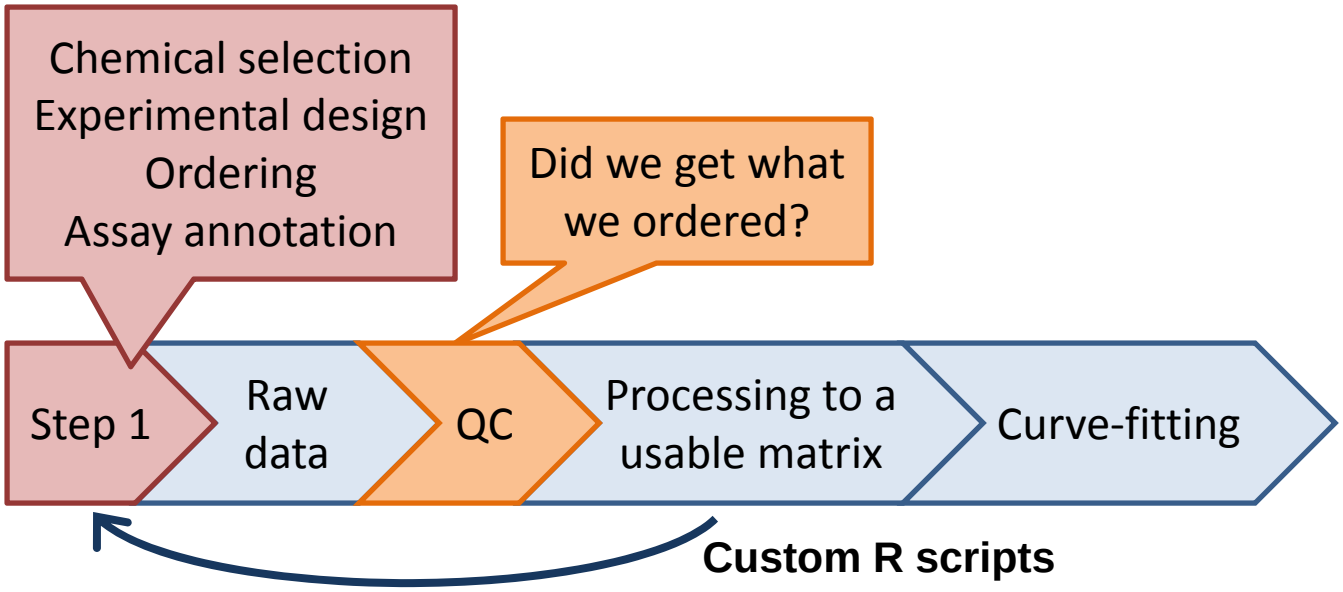


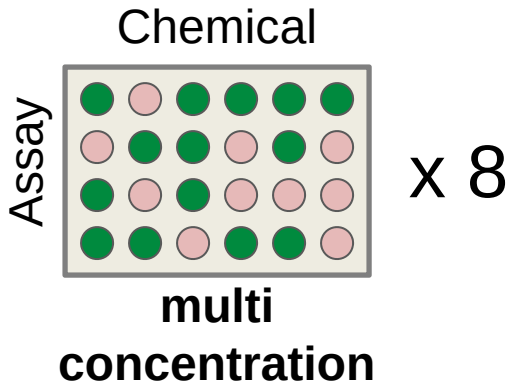
single concentration

323,056
chemical-assay pairs

NovaScreen Workflow

-Caliper Life Sciences (a PerkinElmer company)





x 8

24,118
chemical-assay pairs

Example Chemical-Response Plot

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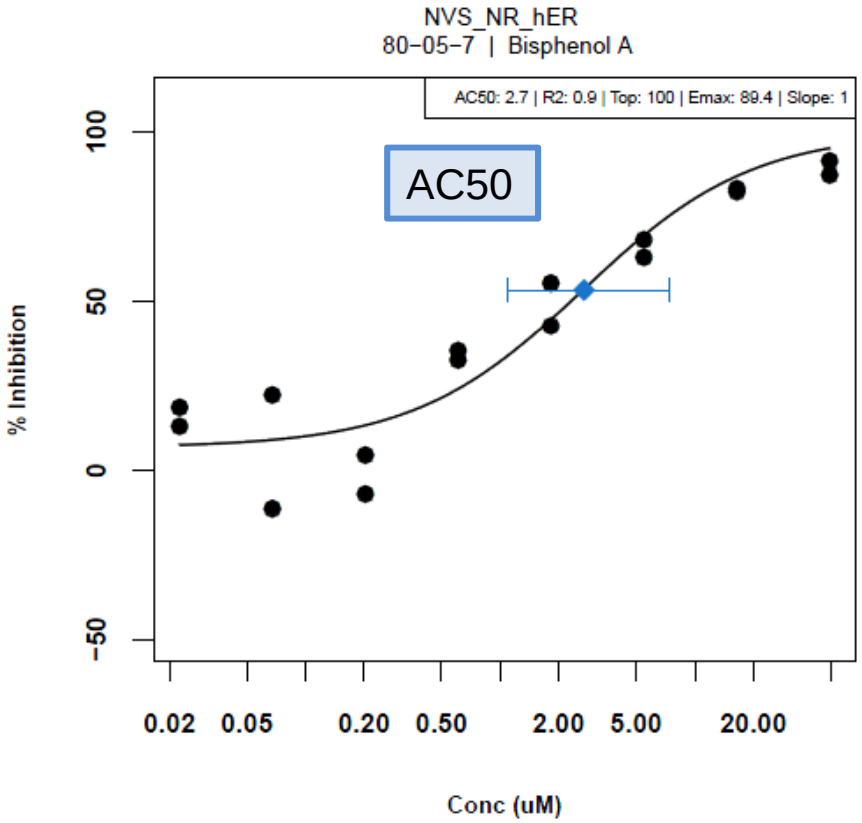


Assay
Chemical name
CASRN
AC50
Min conc tested
Max conc tested
Emax
Slope
R-squared
Fit p-value

7,135 chemical-assay pairs

- **75% chemicals (729/976)**
- **77% assays (256/331)**

Sipes et al 2013, CRT

NovaScreen Workflow

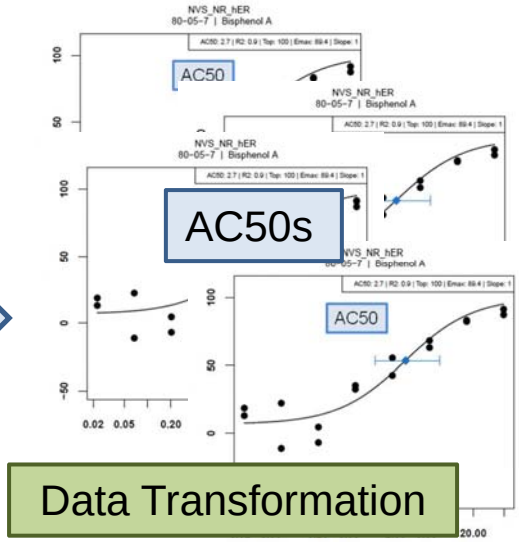
-Caliper Life Sciences (a PerkinElmer company)

Chemical selection
Experimental design
Ordering
Assay annotation

Did we get what we ordered?

Step 1 → Raw data → QC → Processing to a usable matrix → Curve-fitting

Custom R scripts



Data Transformation

Data Analysis

- Statistical software
- Hierarchical clustering
- Correlation matrices

Data Analysis Workflow

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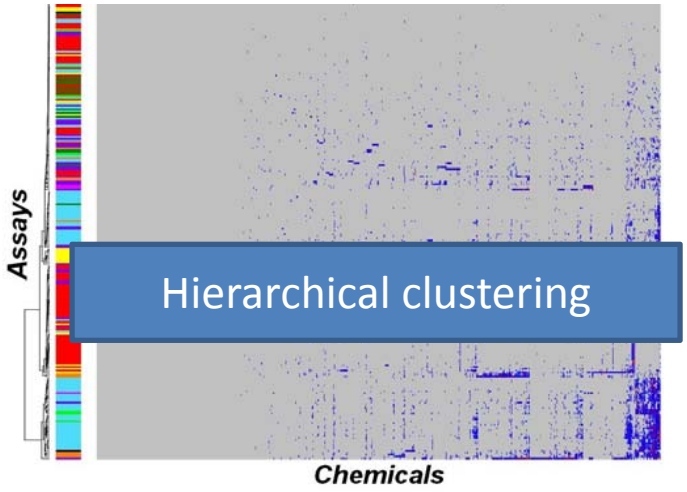
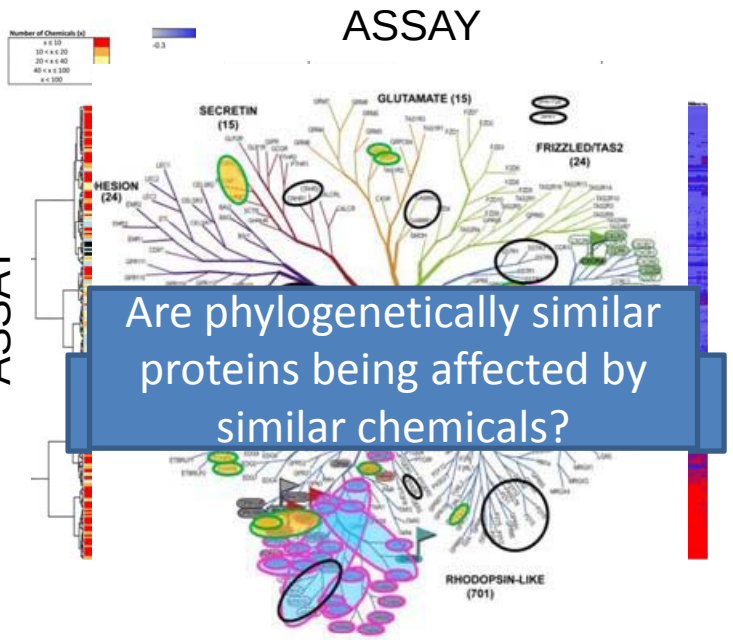
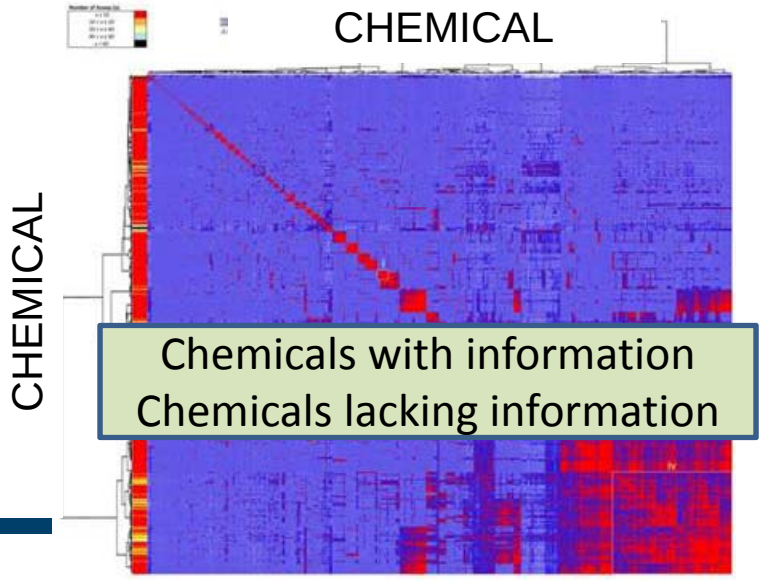


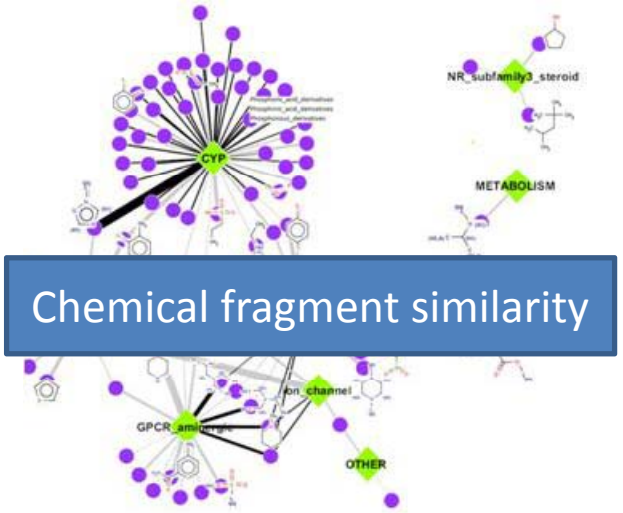
Hierarchical clustering



Are phylogenetically similar proteins being affected by similar chemicals?



Chemicals with information
Chemicals lacking information



Chemical fragment similarity

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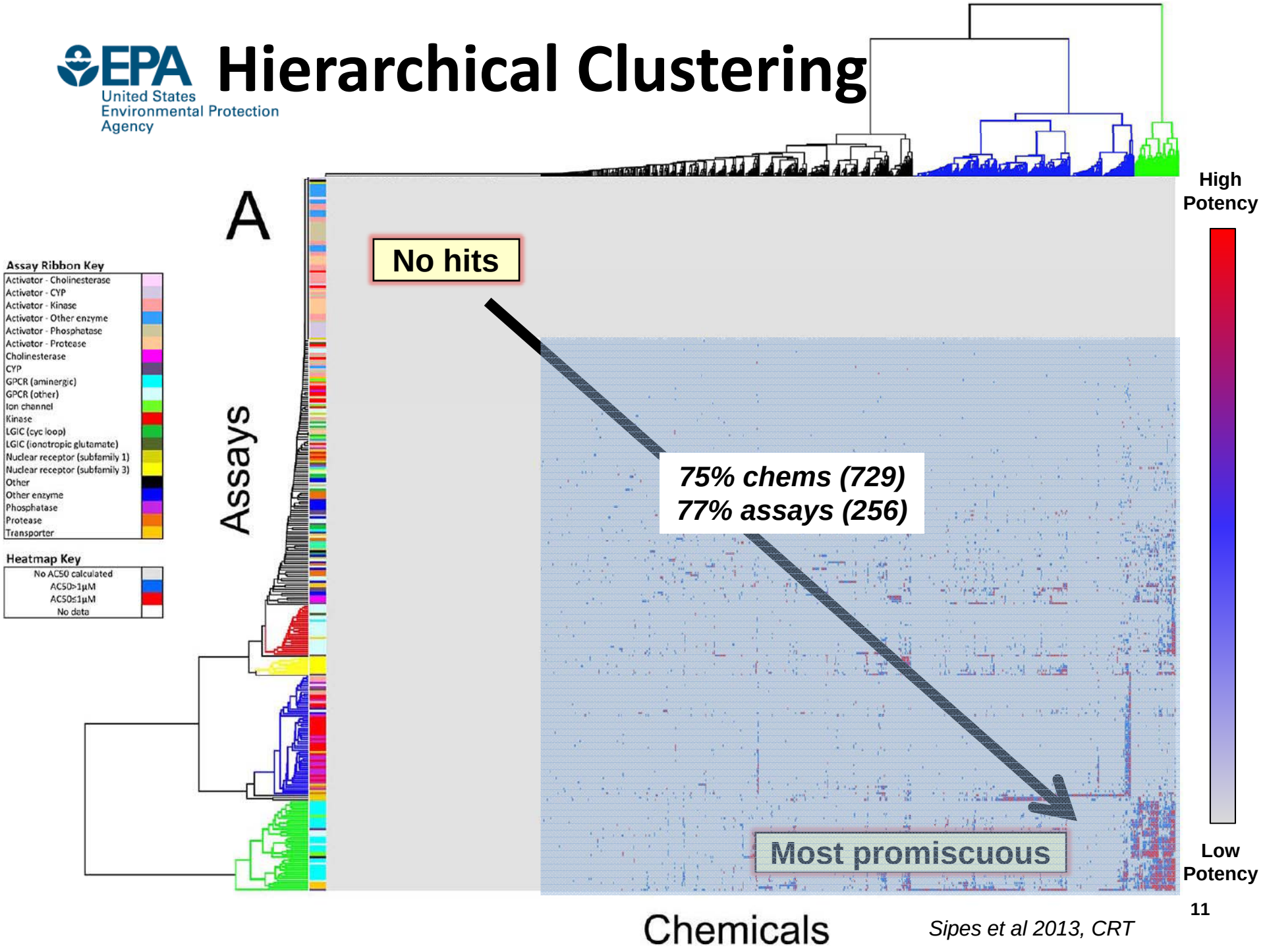
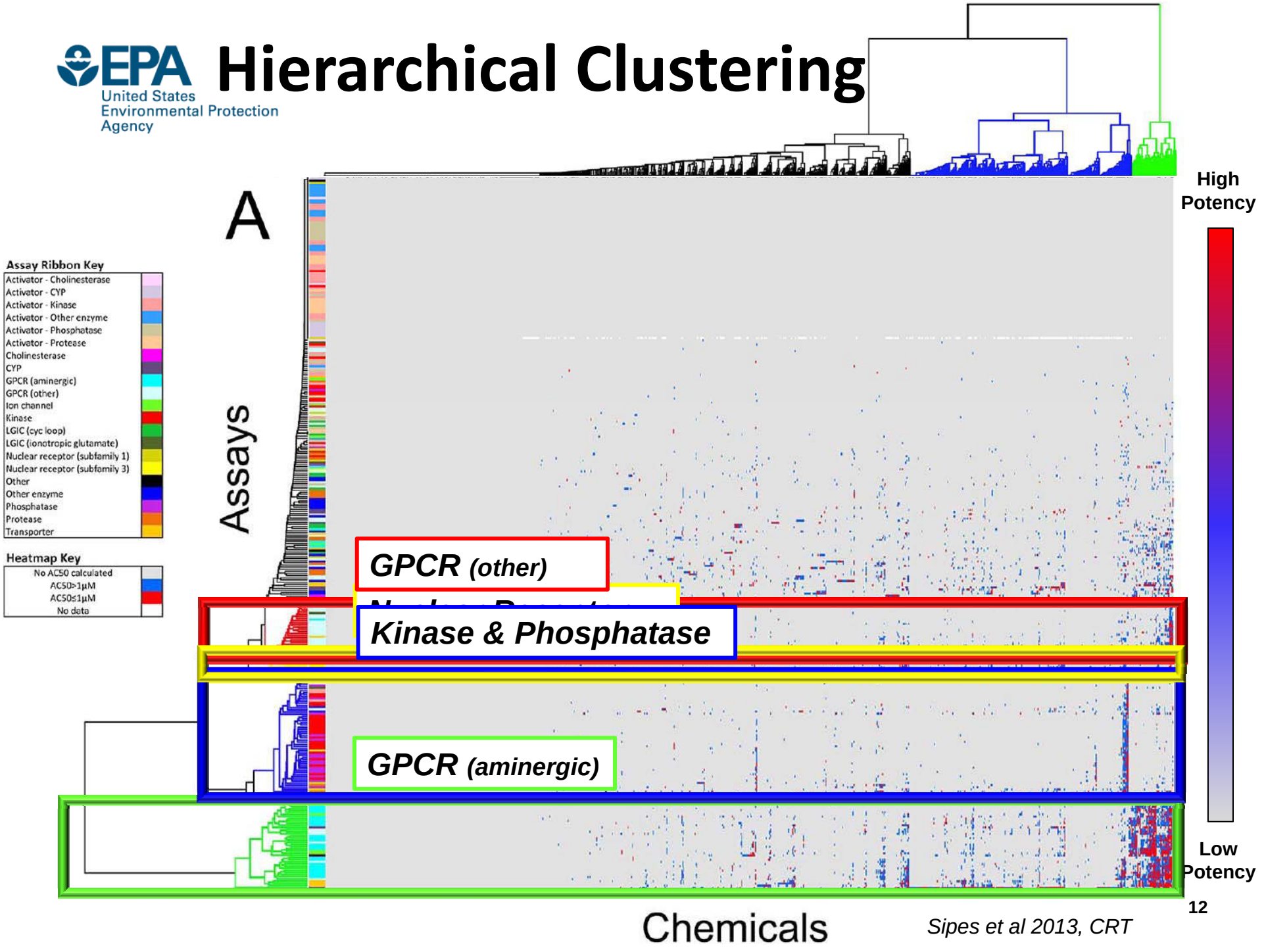
Hierarchical Clustering



Sipes et al 2013, CRT

Hierarchical Clustering

United States Environmental Protection Agency



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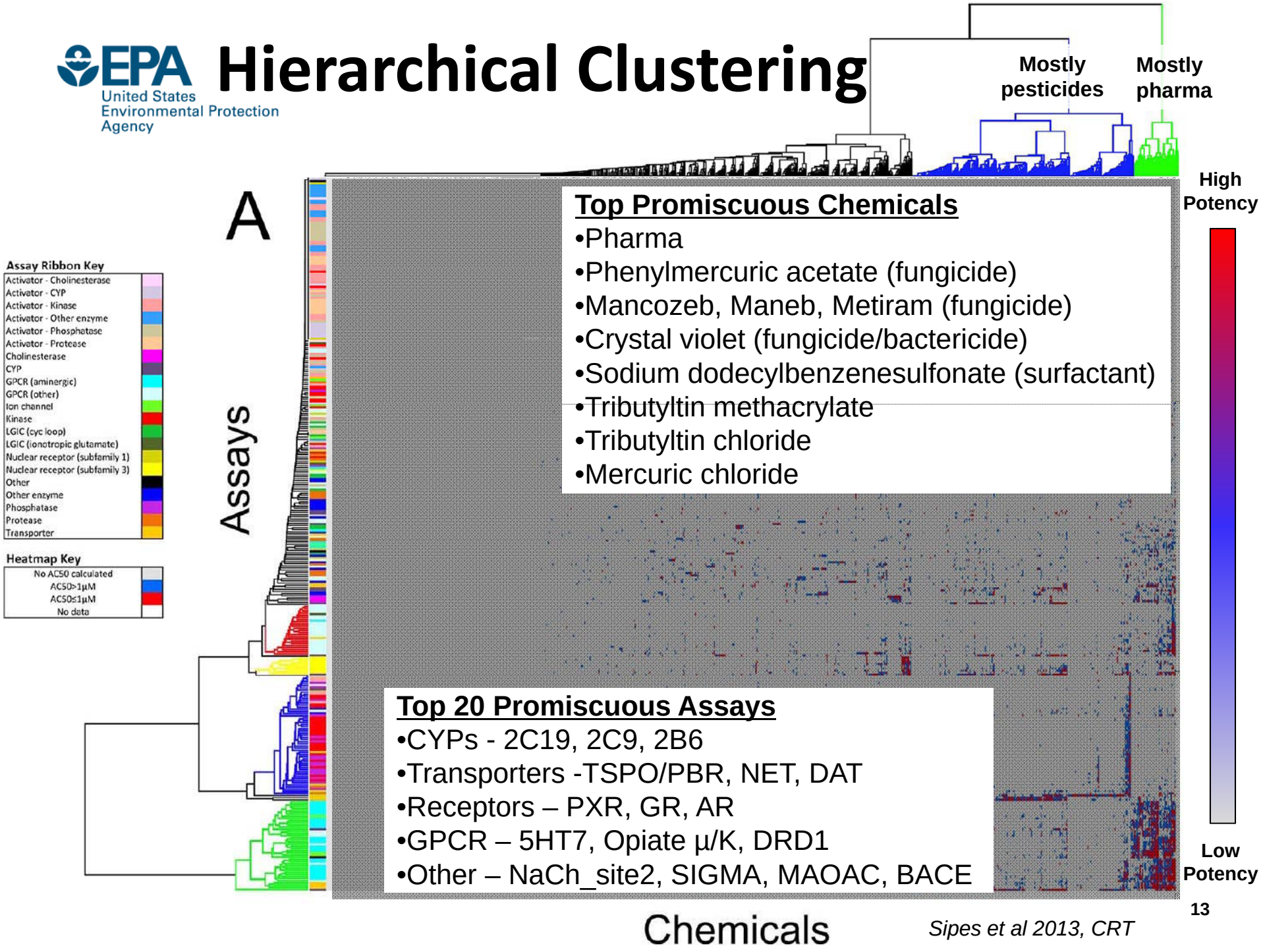
Hierarchical Clustering

United States Environmental Protection Agency



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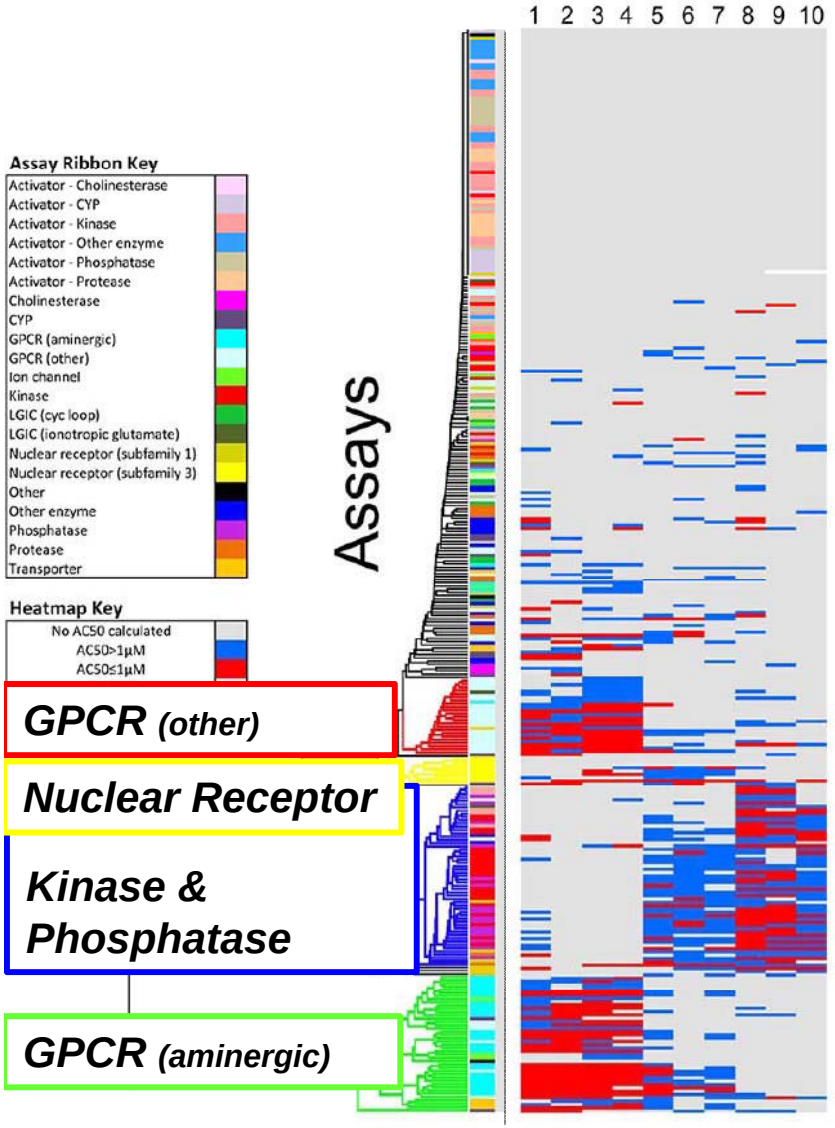
Hierarchical Clustering

United States Environmental Protection Agency

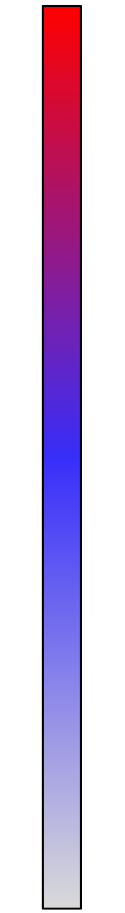


Top Promiscuous Chemicals

Heavy metals
1-Phenylmercuric acetate
2-Mercuric chloride
3-Tributyltin methacrylate
4-Tributyltin chloride

Surfactants
5-Sodium dodecylbenzenesulfonate
6-Perfluorooctane sulfonic acid
7-Dodecylbenzene sulfonate triethanolamine (1:1))

Dithiocarbamate fungicides
8-Mancozeb
9-Maneb
10-Metiram



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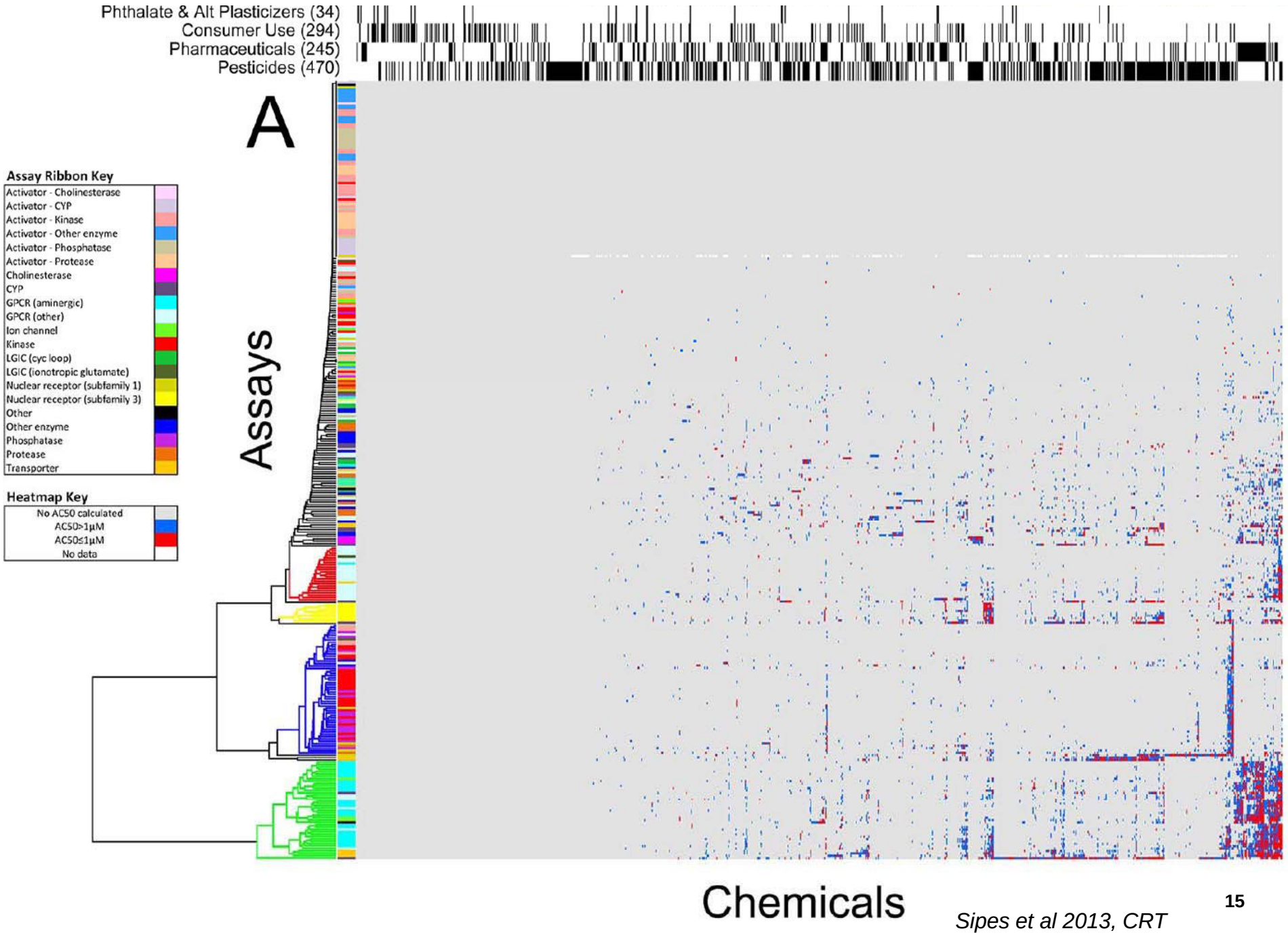

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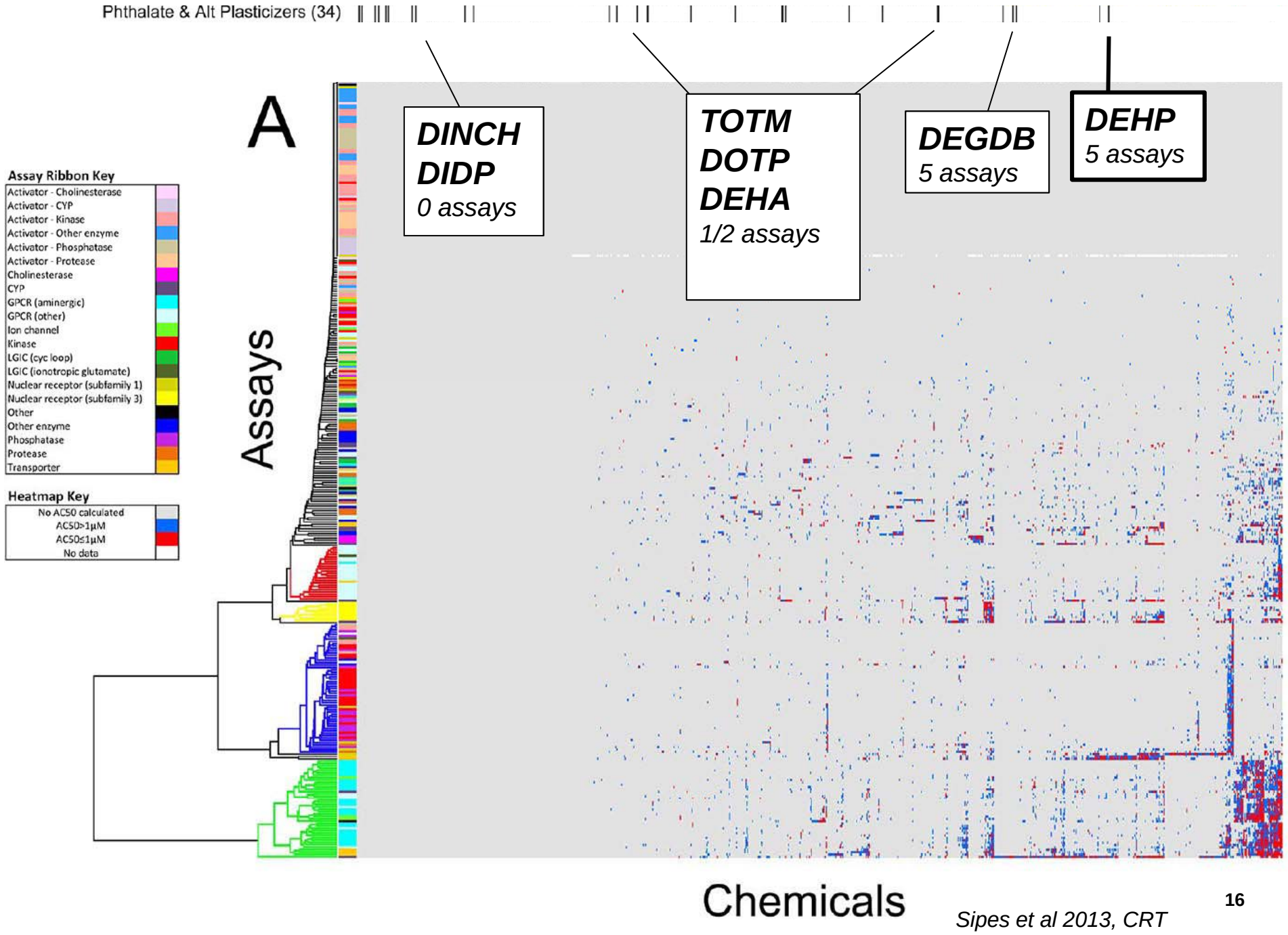

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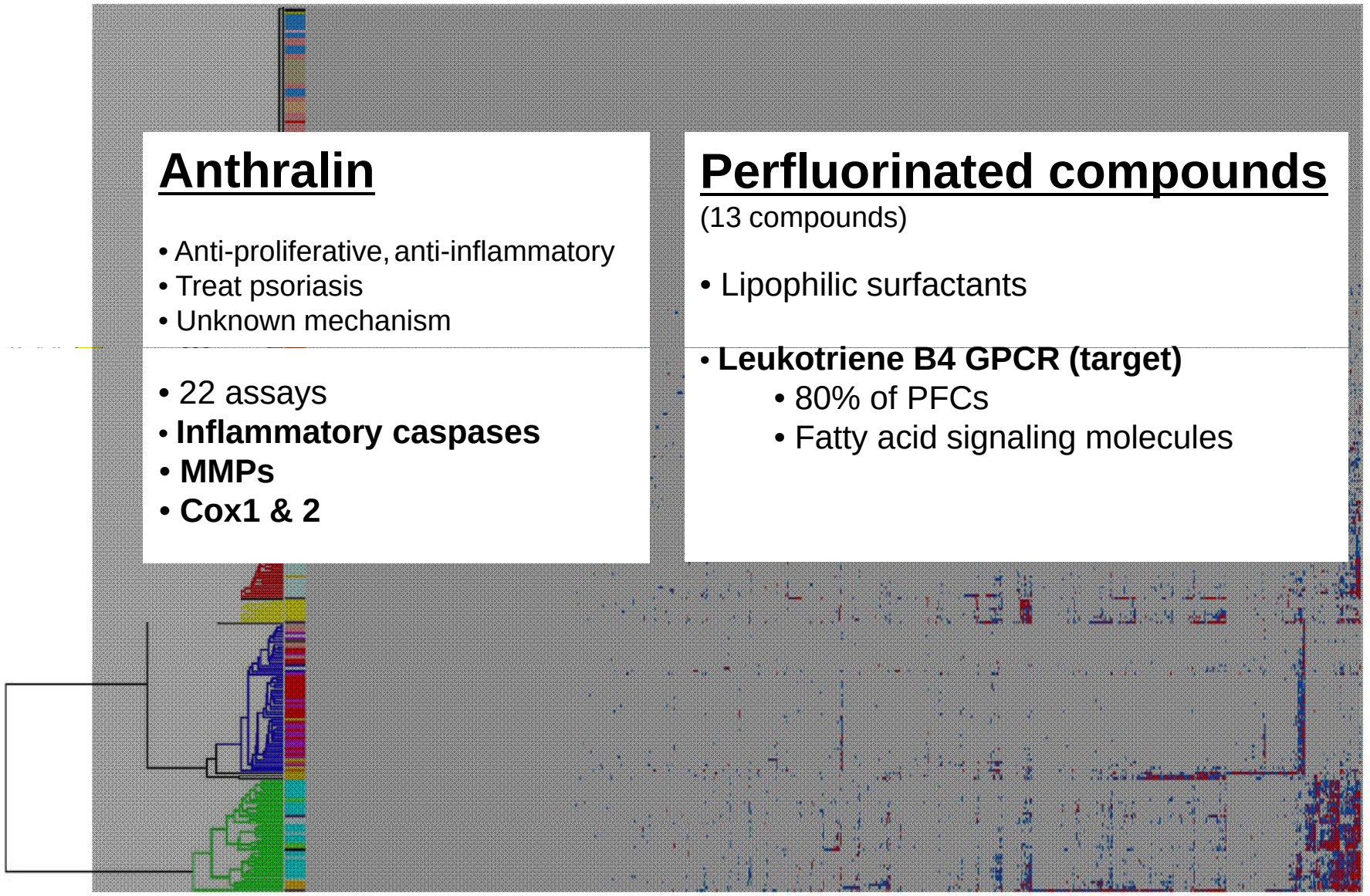

Anthralin

- Anti-proliferative, anti-inflammatory
- Treat psoriasis
- Unknown mechanism

- 22 assays
- **Inflammatory caspases**
- **MMPs**
- **Cox1 & 2**

Perfluorinated compounds

(13 compounds)

- Lipophilic surfactants

- **Leukotriene B4 GPCR (target)**
  - 80% of PFCs
  - Fatty acid signaling molecules

Chemicals

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Most Potent Chemical-Assay Pairs

Table 4. Potent Chemical–Assay Pairs with AC50s at the Lowest Dose Tested[a] AC50s @ 23nM (9nM for CYPs)

chemical name	AC50s total	AC50s LCT[b]	assay target(s)	refs
chlorpromazine hydrochloride	55	7	TR_hNET, GPCR_hDRD1/2s, p5HT2C, hH1, rAdra1A/1B	55–58
haloperidol	42	6	OR_gSIGMA_NonSelective, GPCR_hDRD1/2s/4.4, GPCR_rAdra1_NonSelective, GPCR_bDR_NonSelective	59–61
trelanserin (SL650472 pharma)	24	6	GPCR_h5HT2A/7, p5HT2C, r5HT_NonSelective, GPCR_hDRD1/4.4	62
17β-estradiol	9	5	NR_hAR, bPR, mERa, hER, bER	63,64
17α-ethinylestradiol	24	4	NR_hAR, mERa, hER, bER	65
CP-471358 pharma	6	3	ENZ_hMMP13/2/9	66
CP-544439 pharma	10	3	ENZ_hMMP13/2/9	67
diethylstilbestrol	31	3	NR_mERa, hER, bER	68
2,2-bis(4-hydroxyphenyl)-1,1,1-trichloroethane	36	3	NR_mERa, bER, hCAR_Antagonist	69
zamifenacin (pharma)	60	3	GPCR_gMPeripheral_NonSelective, hM3/5	70,71
flufenacet	10	2	MP_rPBR, NR_hPXR	
maneb	62	2	ENZ_hPTPN9/4	
methadone hydrochloride	37	2	GPCR_rOpiate_NonSelective/Na	72
progesterone	11	2	NR_hAR, NR_bPR	73
SR144190 pharma	24	2	GPCR_hNK2, NR_hPXR	74

- >50% had literature evidence for these associations
  - limited by publically available information
- 20% had literature evidence for additional chemical-target associations missed
  - bioavailability, conc not high enough, species differences, single screen miss

Sipes et al 2013, CRT

Assessing Reliability EDSP Chemicals

Table 5. Known Estrogenic and Nonestrogenic Compounds[a]

relative potency	chemical name	AC50s (μM) bER	hER	mERa
inactive	atrazine	0	0	0
inactive	linuron	0	0	0
inactive	haloperidol	0	0	0
inactive	phenobarbital sodium salt	0	0	0
inactive	progesterone	0	0	0
inactive	ketoconazole	0	0	0
	inactive summary	100%	100%	100%
very weak	ethylparaben	0	0	0
very weak	methoxychlor	0	0	0
very weak	butyl benzyl phthalate	0	0	0
	very weak summary	0%	0%	0%
weak	4-(1,1,3,3-tetramethylbutyl) phenol	33.000	7.200	8.200
weak	kepone	0	0	0
weak	genistein	0.130	0.032	0.130
weak	4-cumylphenol	16.000	0	12.000
weak	bisphenol B	0.430	0.300	0.023
weak	*o,p*-DDT	0	0	0
weak	bisphenol A	0.630	0.820	1.100
weak	4-nonylphenol, branched	33.000	20.000	5.600
weak	butylparaben	56.999	17.000	23.000
	weak summary	78%	67%	78%
strong	17β-estradiol	0.023	0.023	0.023
strong	diethylstilbestrol	0.023	0.023	0.023
strong	17α-ethinylestradiol	0.023	0.023	0.023
	strong summary	100%	100%	100%
antagonist	tamoxifen	0.100	0.330	0.200
	antagonist summary	100%	100%	100%

EDSP reference chemicals

22 chemicals

bovine, human, mouse ER

- inactives (6) 100%
- very weak (3) 0%
- weak (9) 78%
- strong actives (3) 100%
- antagonists (1) 100%

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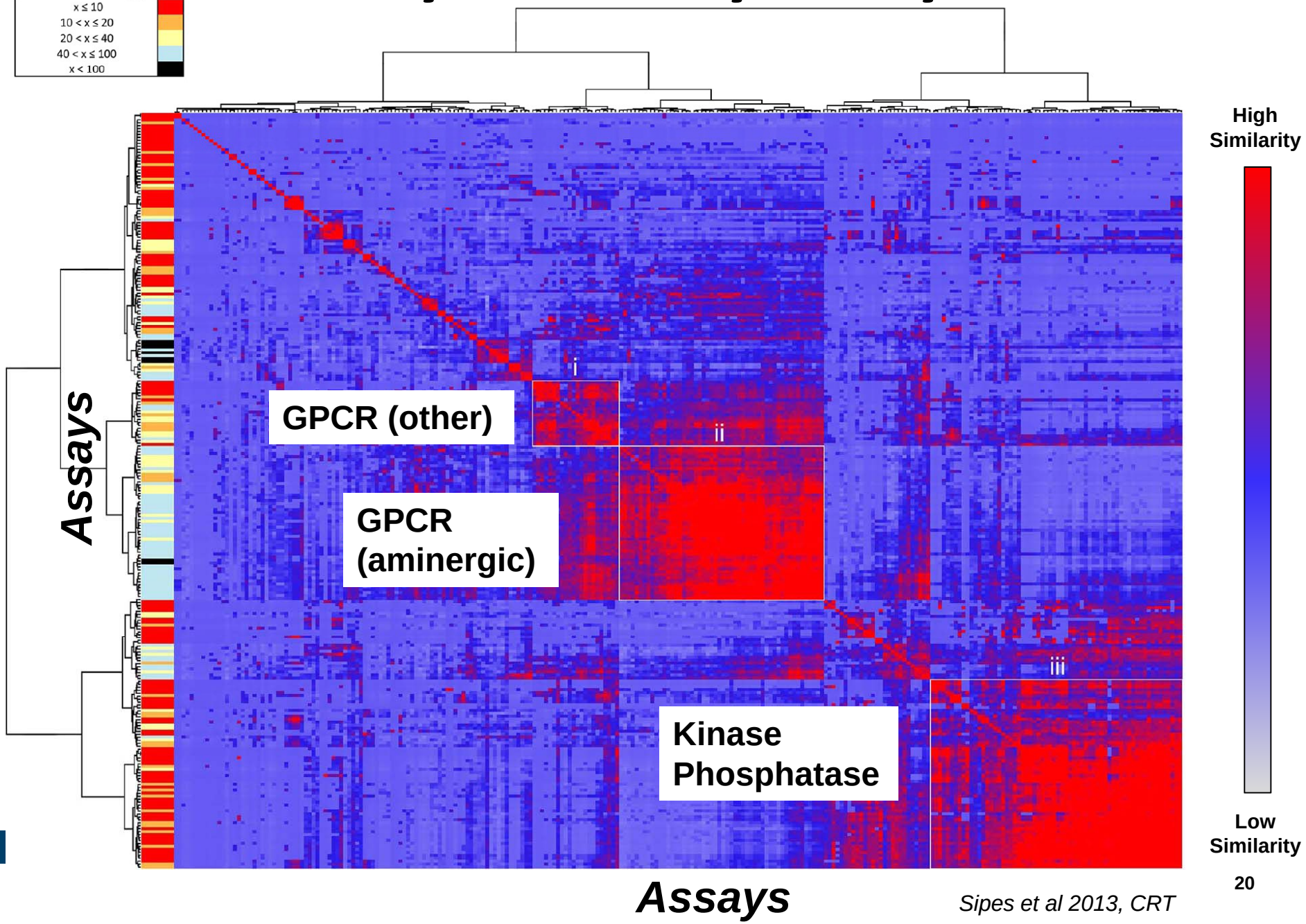
Assay Similarity Analysis



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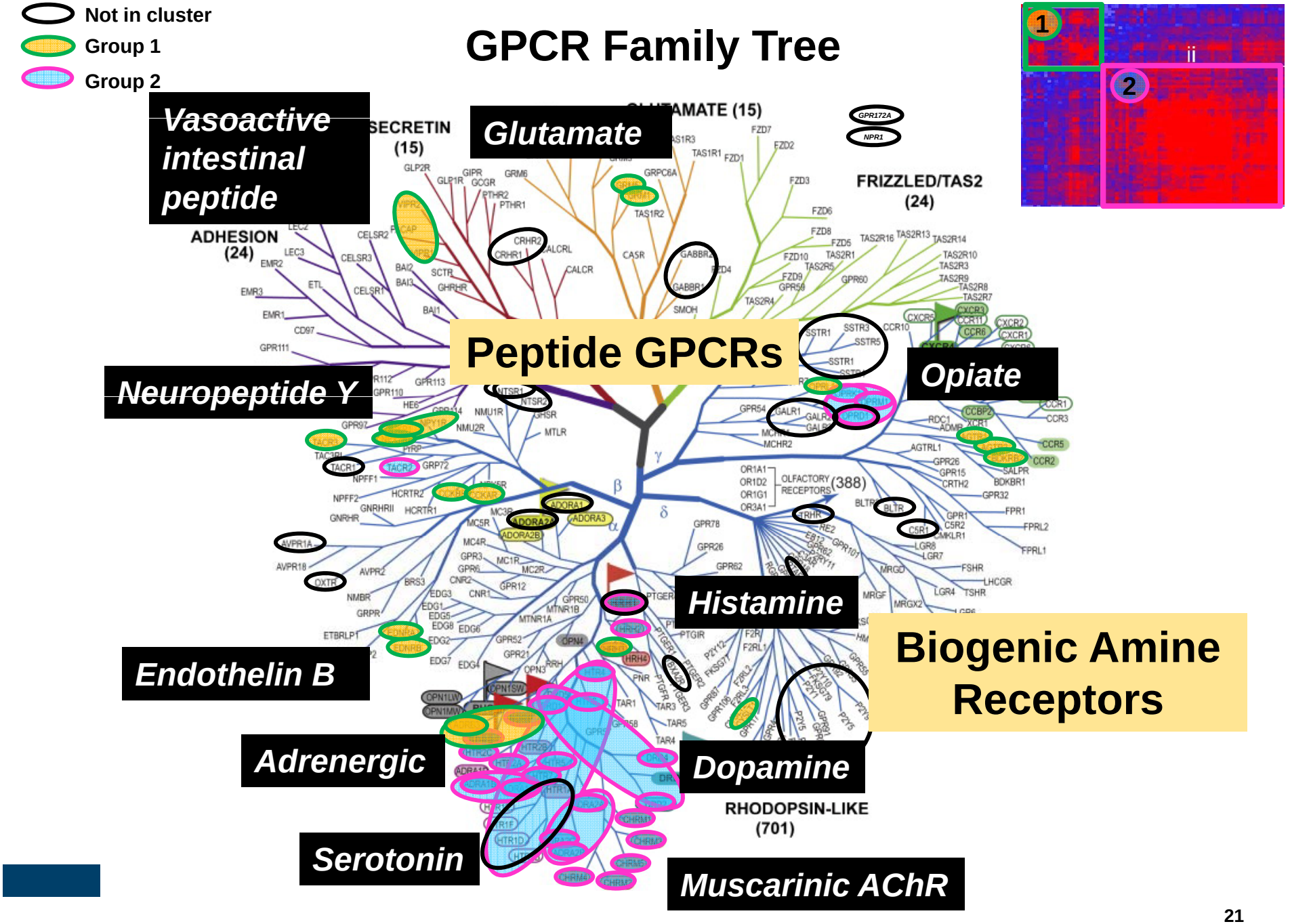
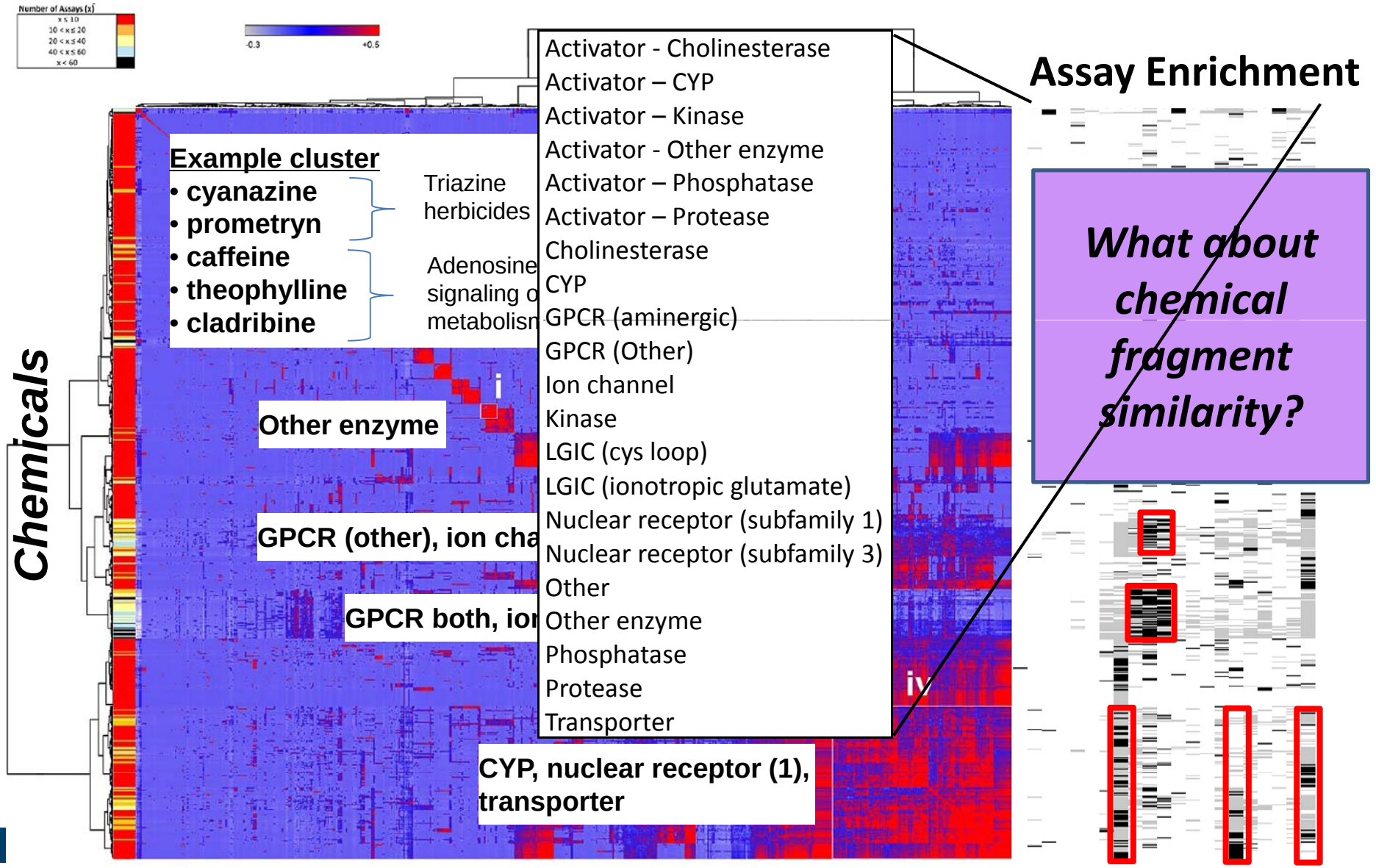
GPCR Family Tree



http://dx.doi.org/10.1016/j.str.2011.05.012

Chemical Similarity Analysis



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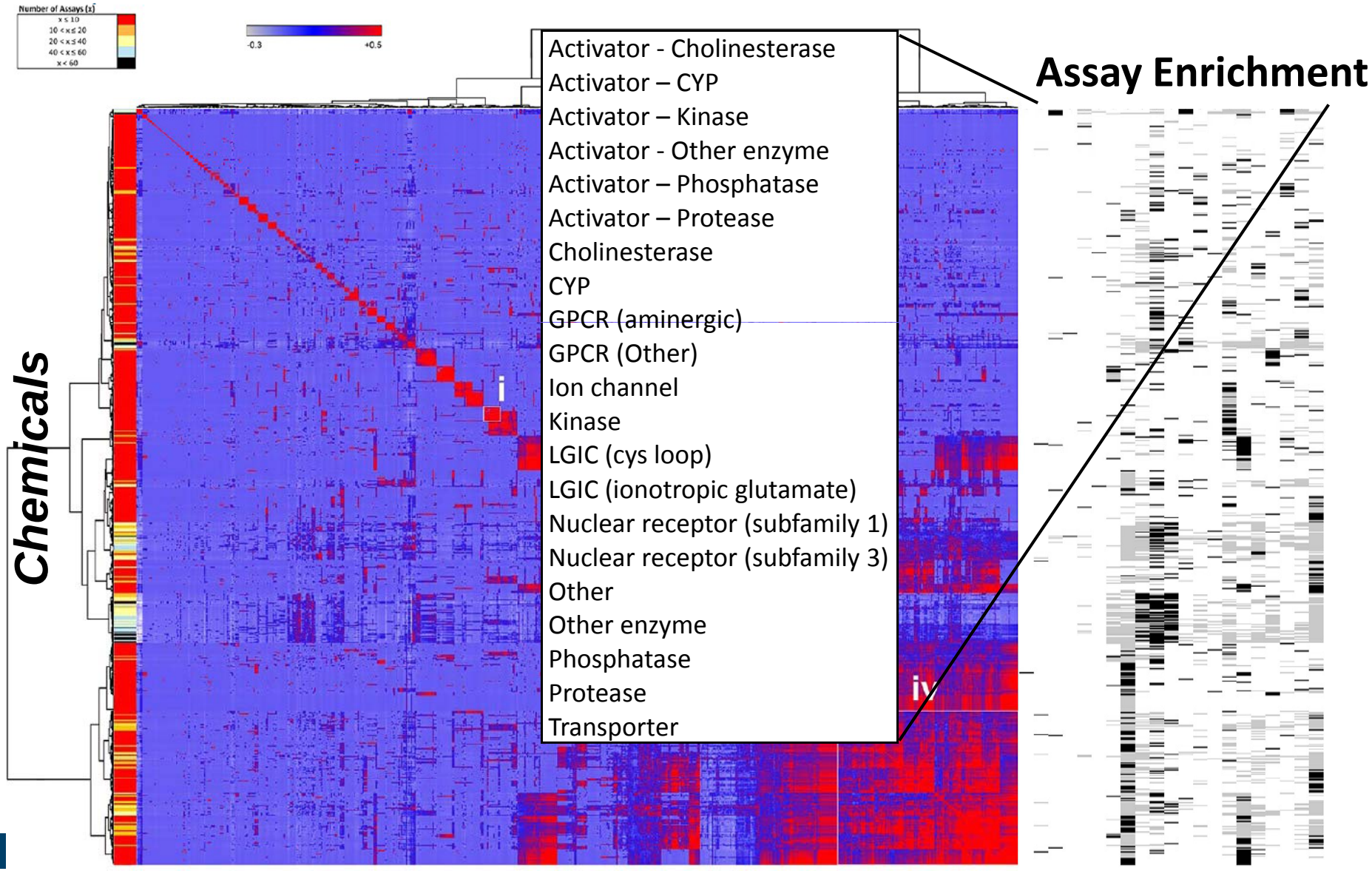
Chemical Similarity Analysis



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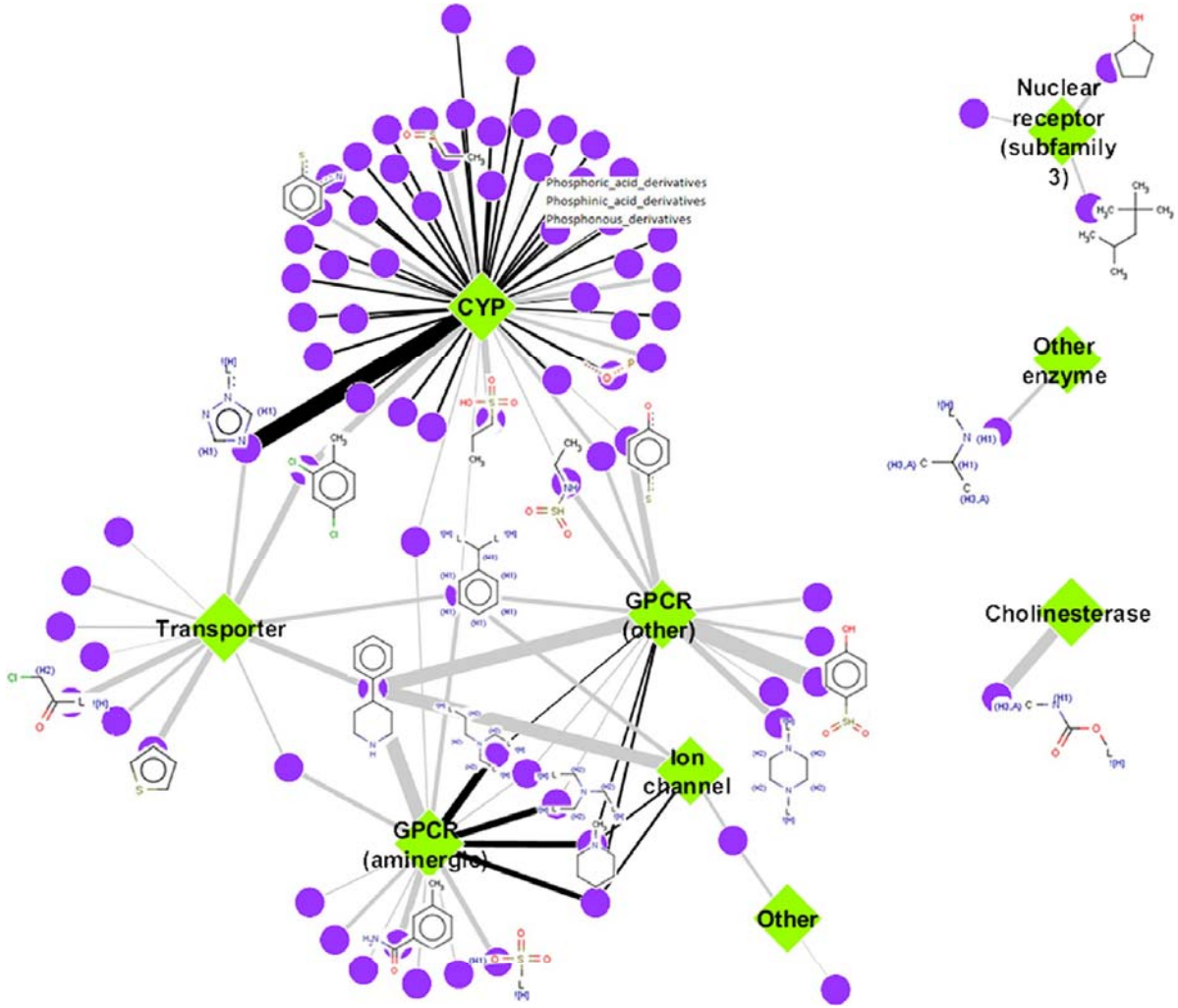
Chemical Fragment-Assay Category Associations



- Simply a description of the features within chemicals preferentially affecting these assay groups
- Fragments are not indicating causal association
- Better inform chemical structure models

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Summary

- ToxCast Phase I & II includes biochemical assay data for 1000 chemicals in >300 assays
- Unique dataset can be used to evaluate additivity of effects across concentration range in combination with cell-based data
- Associations may help inform chemical structure models for predicting chemical-target interactions.
- A combination of these *in vitro* results along with *in vivo* toxicity data are being used in building predictive models for chemical prioritization

Thank you!

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http://pubs.acs.org/doi/abs/10.1021/tx400021f

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