Determining points of departure from multivariate experimental data

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The views expressed in this presentation are those of the author[s] and do not necessarily reflect the views or policies of the U.S. Environmental Protection Agency.

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I have no conflict of interest with the work included in this presentation.

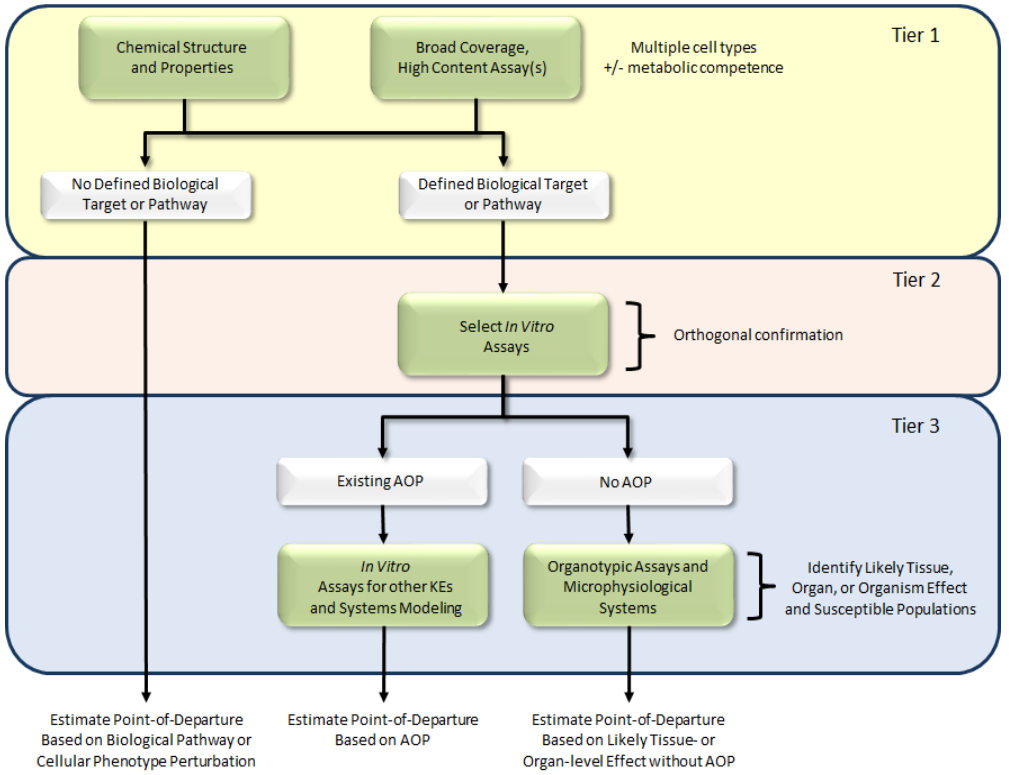
Outline

- New approach methodologies (NAMs)
- High-throughput transcriptomics (HTTr)
- Multivariate analysis via connectivity mapping with signatures
- NAM-based point of departure using signature concentration-response analysis

Tiered Hazard Evaluation

- New approach methodologies (NAMs) are any technology, methodology, approach, or combination of methods that can provide information about chemical hazard and point of departure (POD) without using whole animals
- NAMs based on high-throughput profiling (HTP) assays are flexible, portable and cost-efficient platforms to comprehensively evaluate the potential effects of chemical exposure
- Two types of HTP assays:
  - High-throughput transcriptomics (HTTr)
  - High-throughput phenotypic profiling (HTPP)
- We are evaluating HTPP and HTTr to develop NAMs
  - For hazards: identify putative targets and pathways
  - For POD: estimate *in vitro* POD associated with hazards

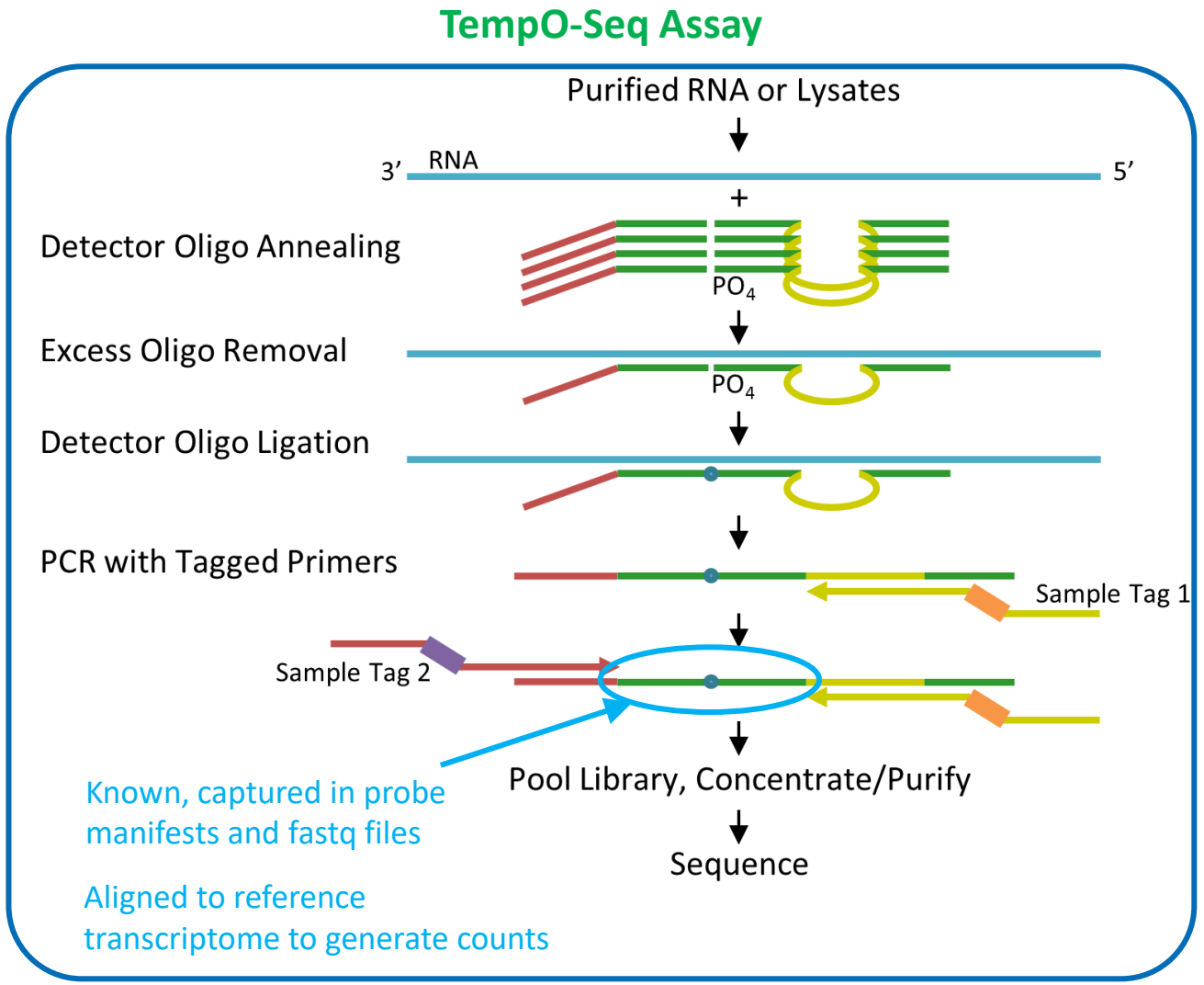
"CompTox Blueprint"



Thomas *et al.*, 2019

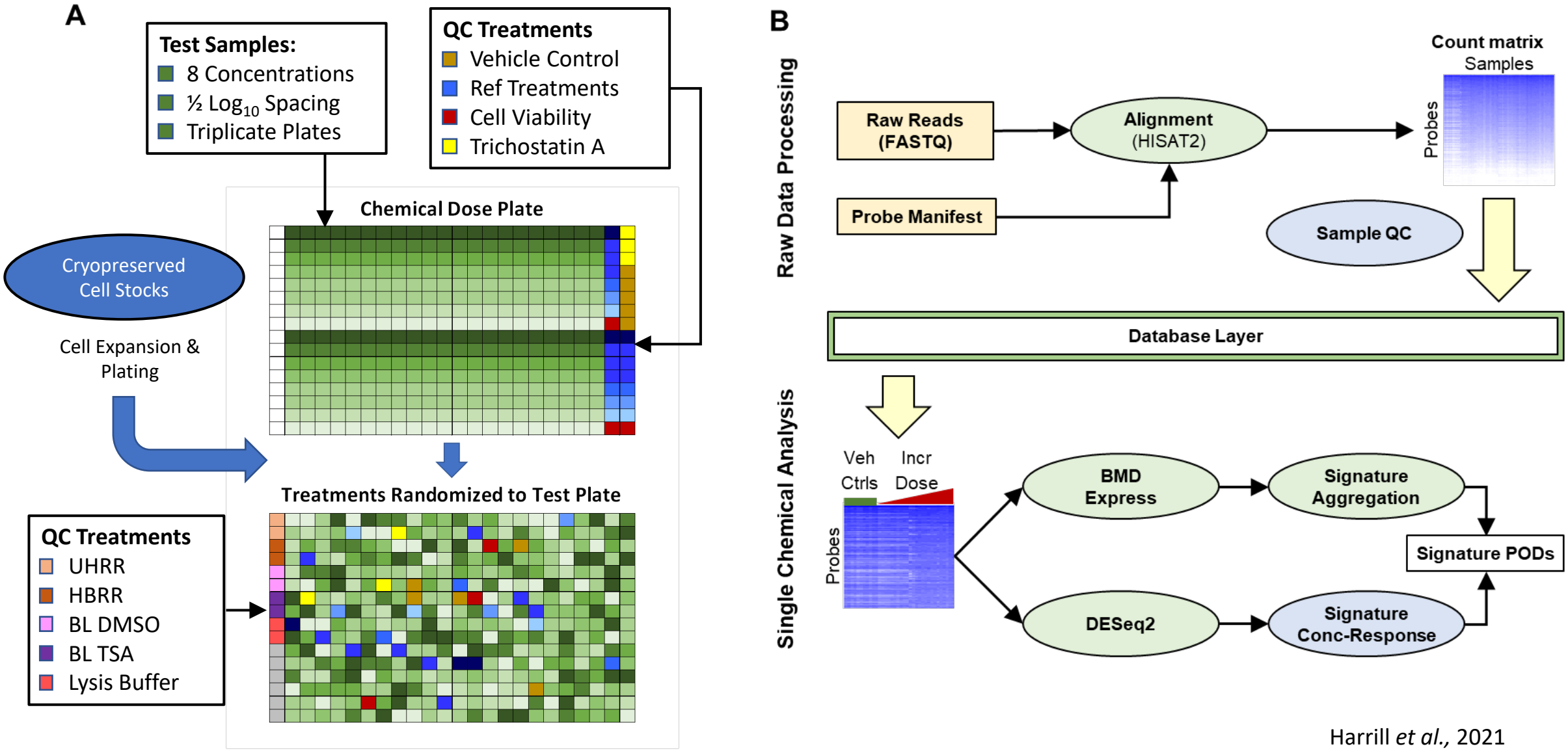
Templated Oligo with Sequencing Readout (TempO-Seq)

- The TempO-Seq human whole transcriptome assay measures the expression of greater than 20,000 transcripts.
- Requires only picogram amounts of total RNA per sample.
- Compatible with purified RNA samples or cell lysates.
- Lysates are barcoded according to sample identity and combined in a single library for sequencing using industry standard instrumentation.
- Scalable, targeted assay:
  - 1) specifically measures transcripts of interest
  - 2) ~50-bp reads for all genes
  - 3) requires less flow cell capacity than RNA-Seq



Yeakley, et al. PLoS ONE 2017

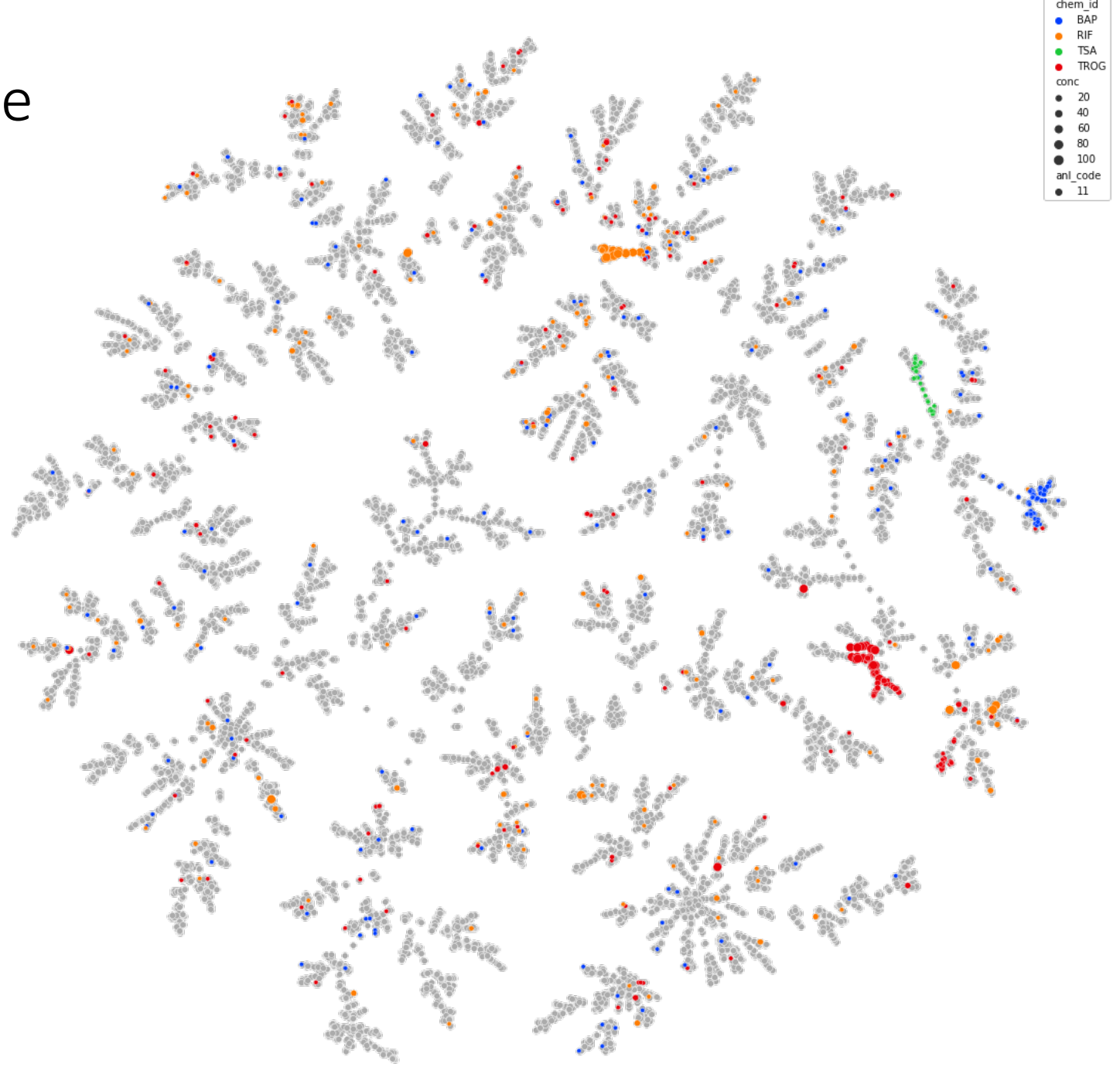
HTTr Experimental Design and Bioinformatics Workflow



Harrill *et al.*, 2021

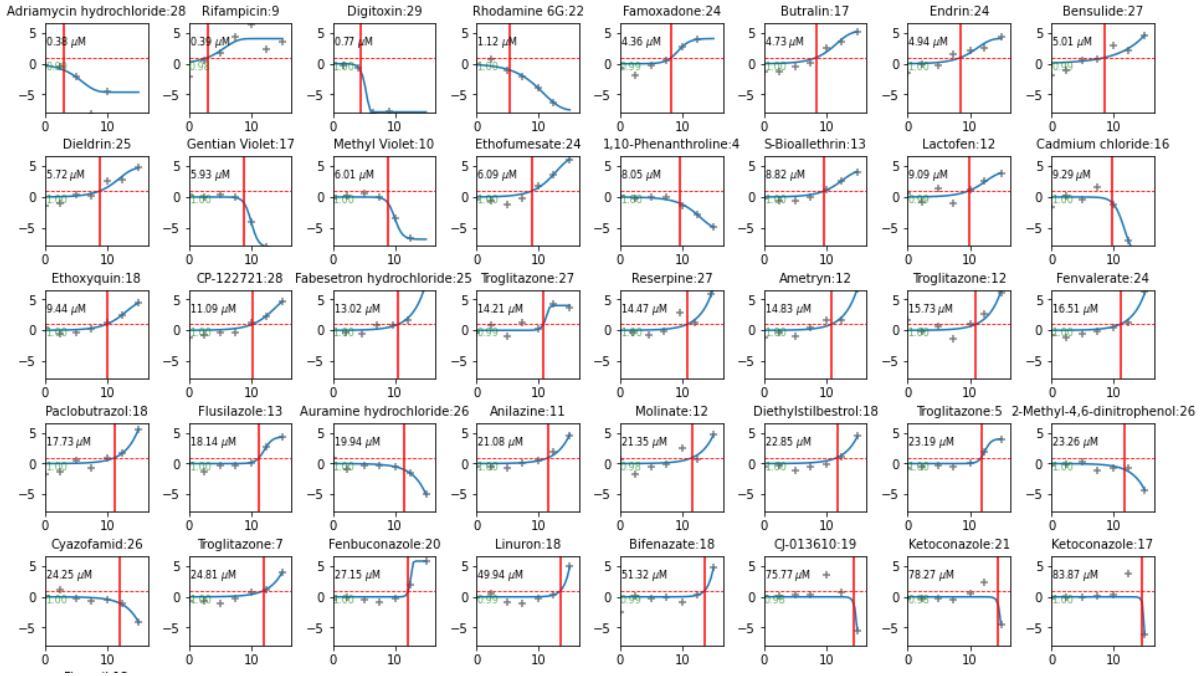
We are generating large-scale HTTr data sets on environmental chemicals

- For example, HepaRG cells treated with 1,366 chemicals 8 concentrations (0.01-100µM) for 24h
- Four reference chemicals: benzo(a)pyrene (●), rifampicin (●), trichostatin A (●) and troglitazone (●)
- Data processed by EPA HTTr pipeline to produce 11,551 L2FC profiles x ~12,250 genes
- Figure shows clustering of all 11,551 transcriptomic fingerprints
- Over 100,000 raw HTTr profiles generated on thousands of chemicals in MCF7, U2OS and other cell types in process

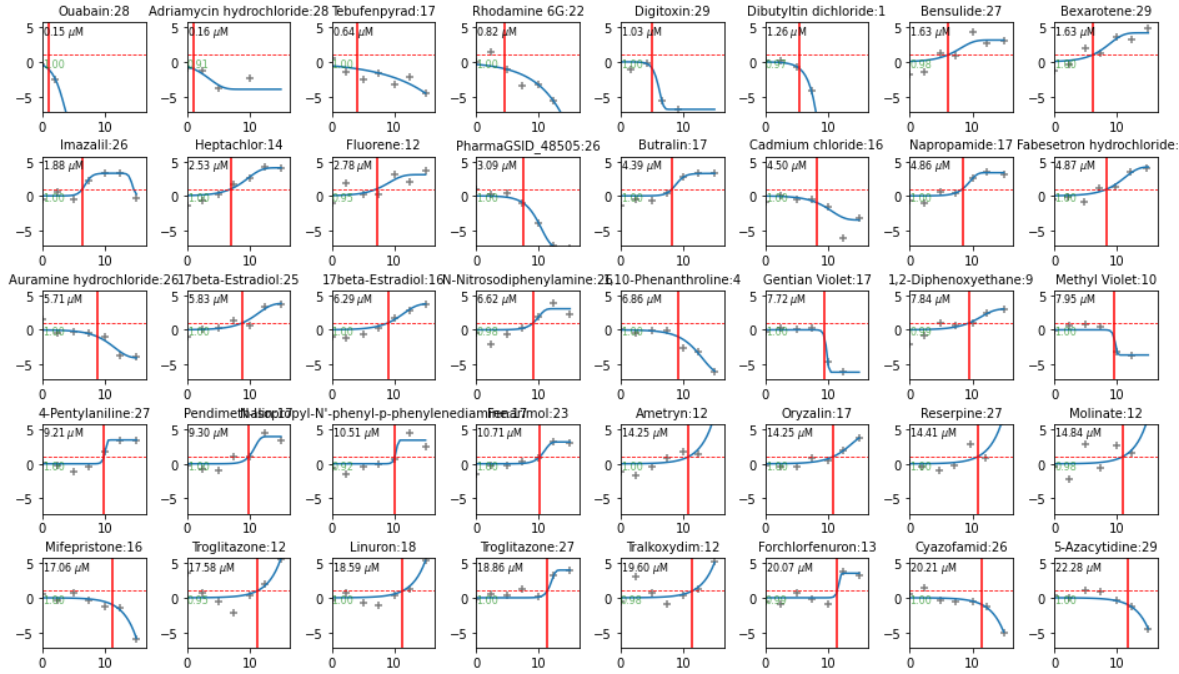


Univariate analysis of each gene is feasible …

PXR/NR1I2: CYP3A4



CAR/NR1I3: CYP2B6

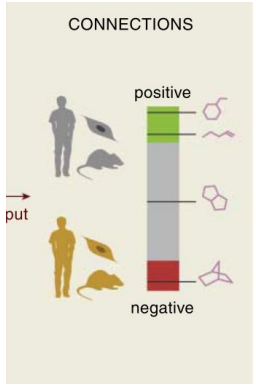


- Used HepaRG L2FC data and R/tcplFit2 (Judson *et al.*) for curve fitting to find benchmark concentrations (BMCs)
- However, this can miss subtle but coordinated regulation of multiple genes in pathways

Multivariate analysis of transcriptomic profiles

Connectivity mapping

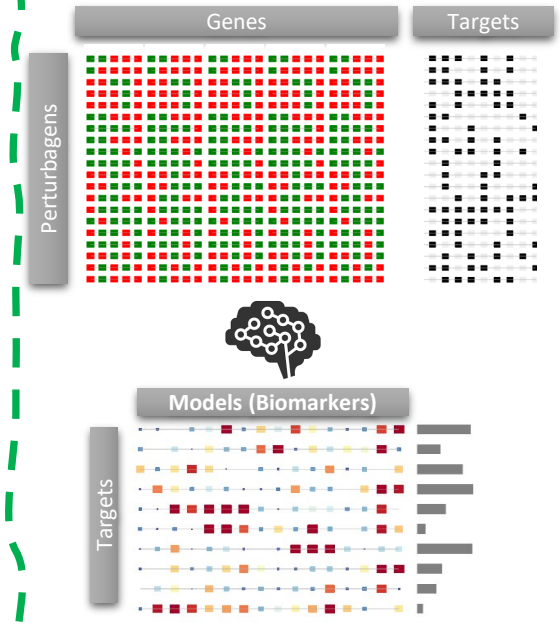
Match test profiles with gene signatures of known mechanisms



Lamb *et al.*, 2006

Machine learning

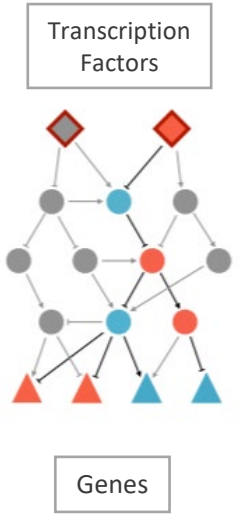
Match profiles with classifiers / biomarkers developed using machine learning



Liu *et al.*, 2017

Causal analysis

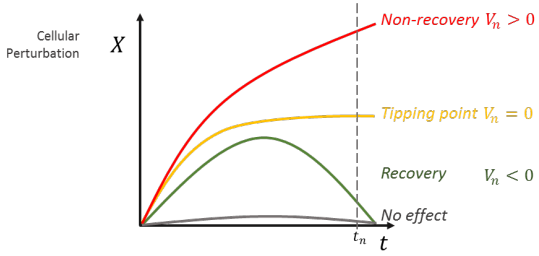
Reconstruct signaling and genetic regulatory interactions to define mechanistic networks



Liu *et al.*, 2019

Tipping Points

Analyze stress response pathway dynamics to estimate critical concentrations at homeostatic thresholds



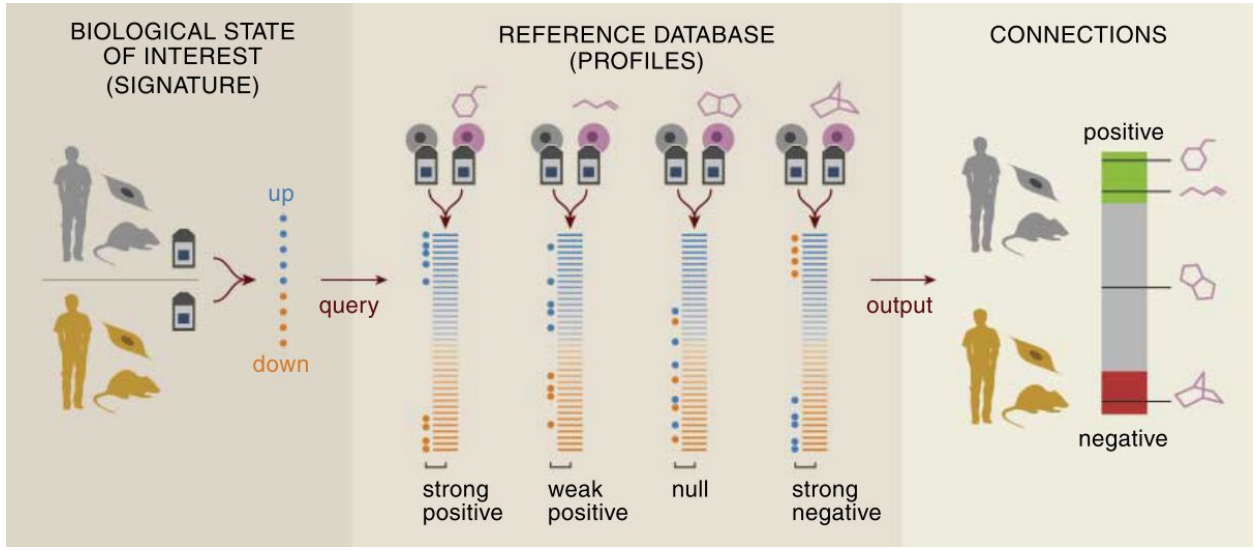
Calculate velocity $V = \frac{dX}{dt}$

Shah *et al.*, 2016

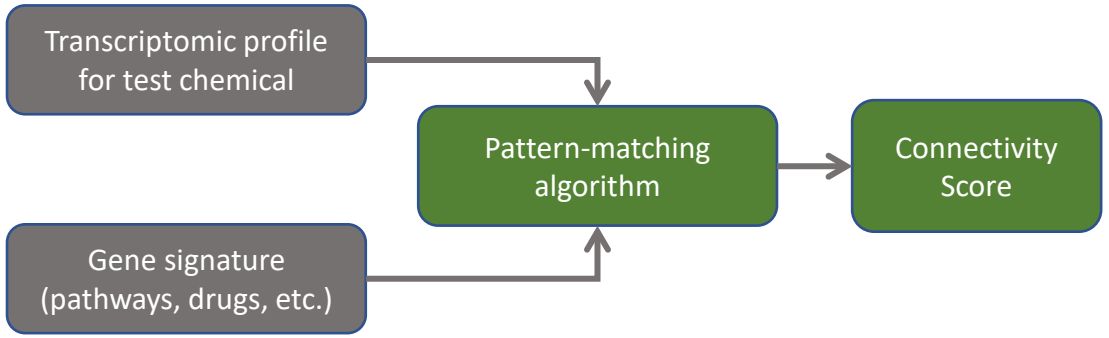
Connectivity Mapping

- "Connectivity mapping" (Lamb *et al.,* 2006): *S*imilarity between transcriptomic profiles may suggest common mechanisms
- Assumes that diverse biological states can be "fingerprinted" by the universal language of genes
- Used for linking chemicals, genes, diseases & drug-repurposing
- Increasing utility in toxicology to find putative targets (DeAbrew *et al.,* 2016; Wang *et al.,* 2016)

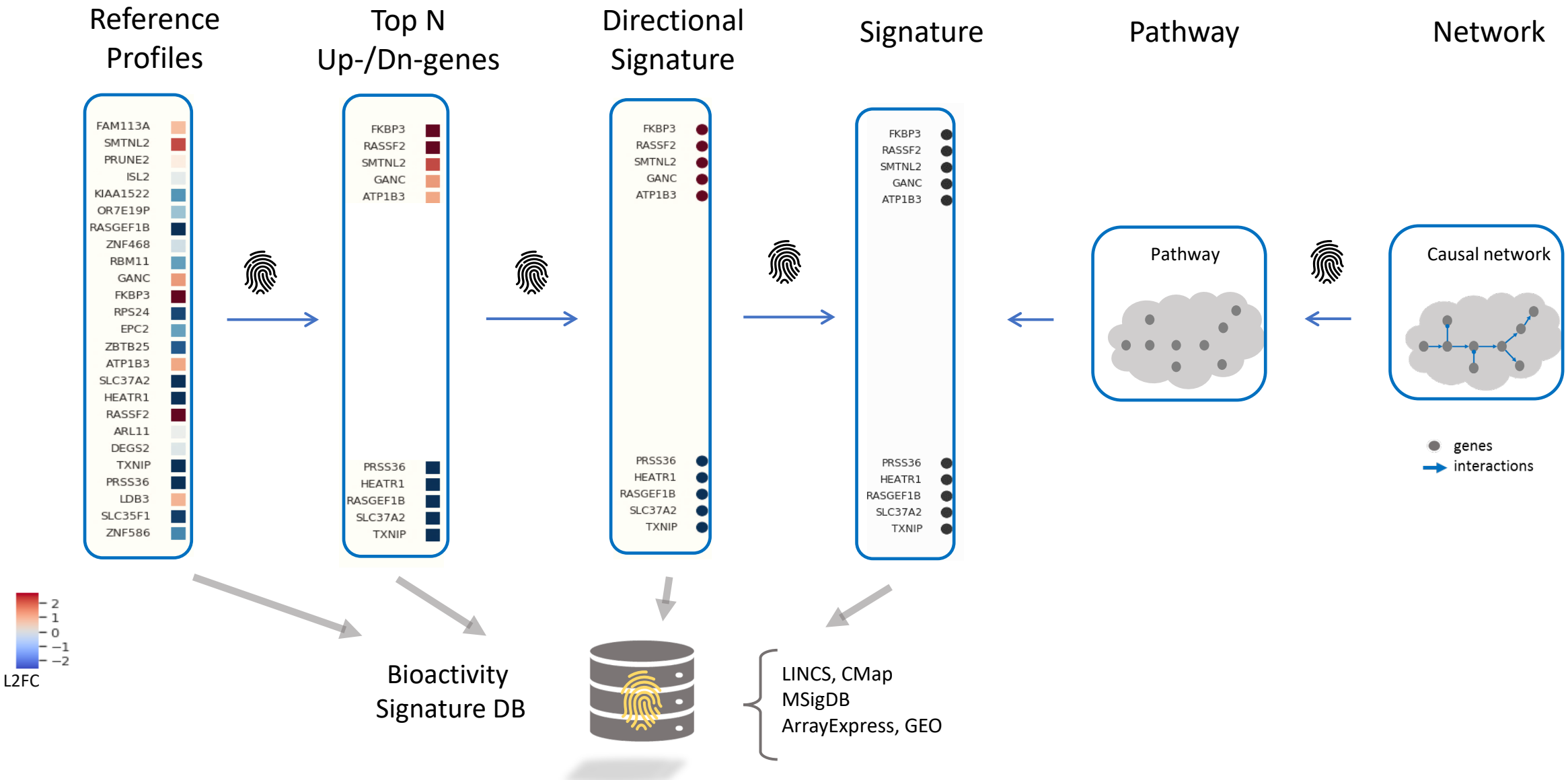
Overview



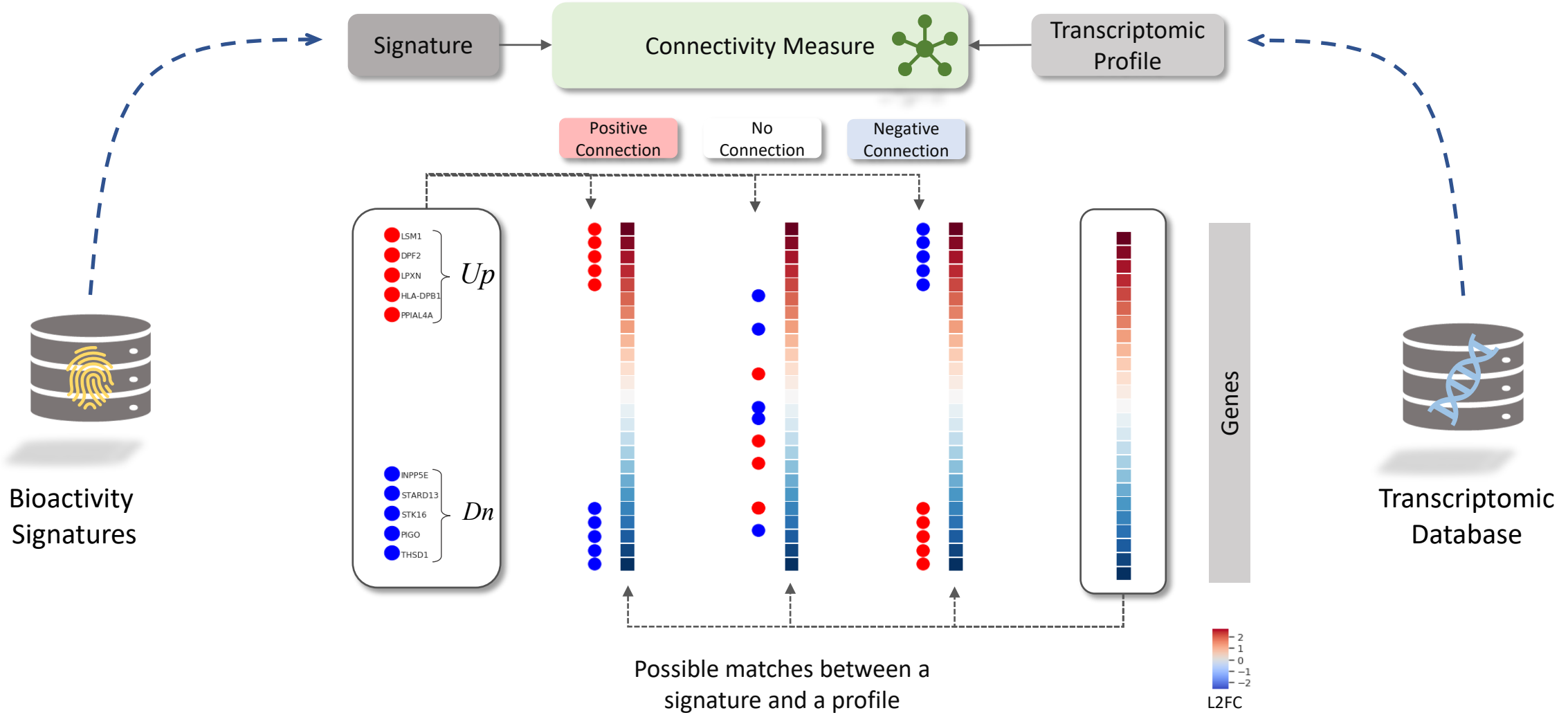
Lamb *et al.,* 2006



“Fingerprinting” bioactivity via gene signatures



Connectivity-mapping with gene signatures



Shah *et al.* (in prep)

Gene Set Connectivity Toolkit (gecco)

DB	Source	Signatures
Srp	US EPA	83
Lincs	Lincs	30,000
cmap	CMap v2	1200
msigdb	MSigDB	26860

- MongoDB for storage
- Consistent document structure
- Supports public and in-house data
- Multiple HTTr technologies

Connectivity Measures

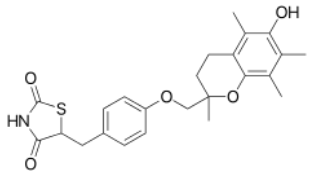
Methods	Measures
Aggregation-based	eXtreme Sum (XS), eXtreme Mean (XM), T-statistic (TT-p), Ranksum statistic (RS), Kolmogorov-Smirnov statistic (GSEA), Total enrichment score (TES)
Vector-based	Extreme Pearson correlation (XCP), Extreme Spearman Correlation (XCS), Jaccard index (JI), Signed Jaccard (SJI), Szymkiewicz–Simpson index (SI), Signed Szymkiewicz–Simpson index (SSI)

- Standardized API in Python 3
- Multiple connectivity measures
- Parallelized for speed
- Uses tcplFit2 for curve-fitting / BMD

DB	Source	Profiles
lincs	Lincs	591697
cmap	CMap v2	6100
arexp	ArrayExpress	3843
mcf7	US EPA	31352
heparg	US EPA	23102
u2os	US EPA	22980

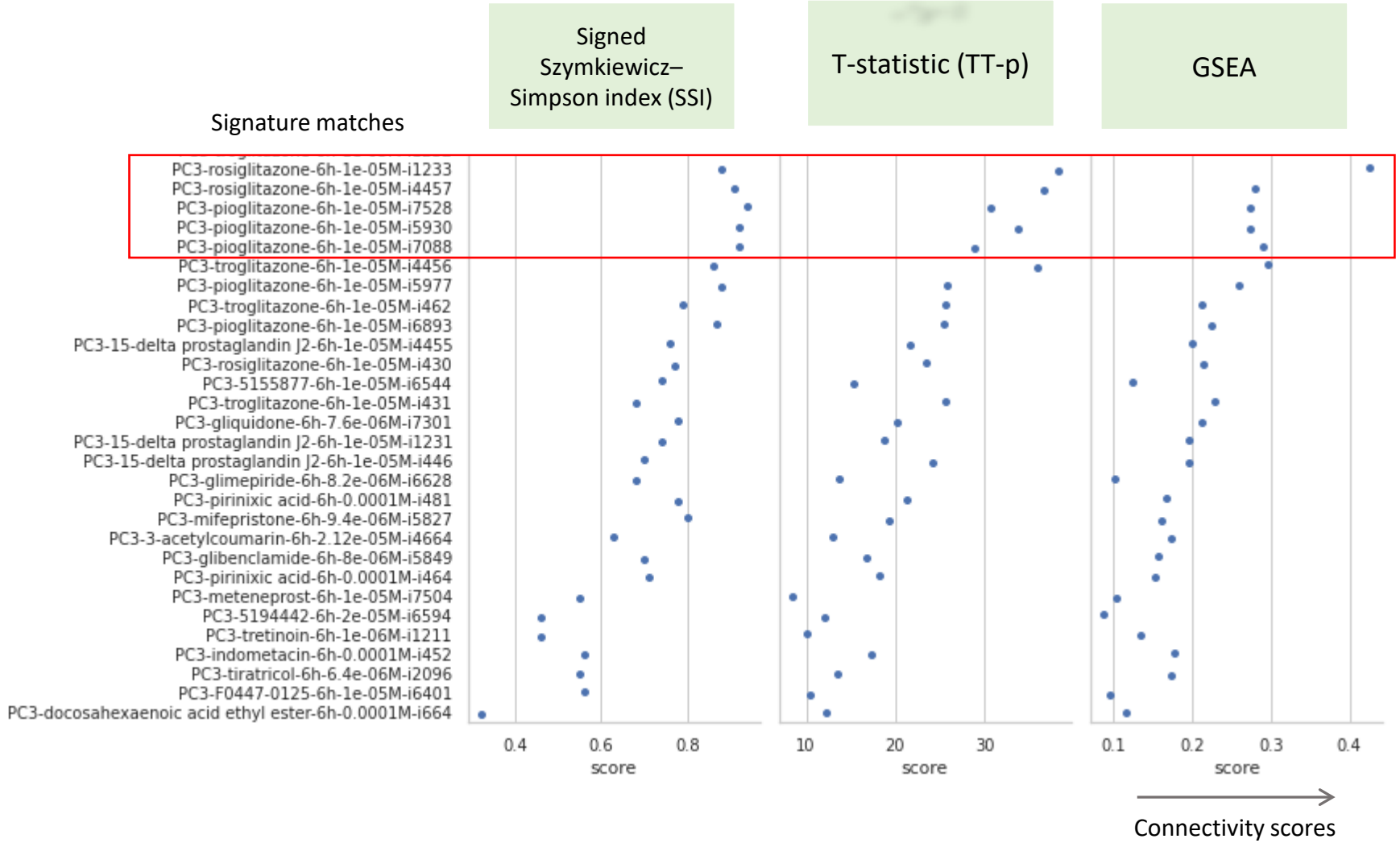
- MongoDB for storage
- Multiple HTTr technologies

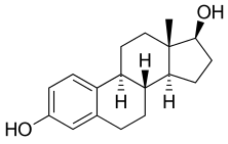
Shah *et al.* (in prep)



Matching troglitazone transcriptomic profiles with other chemical signatures

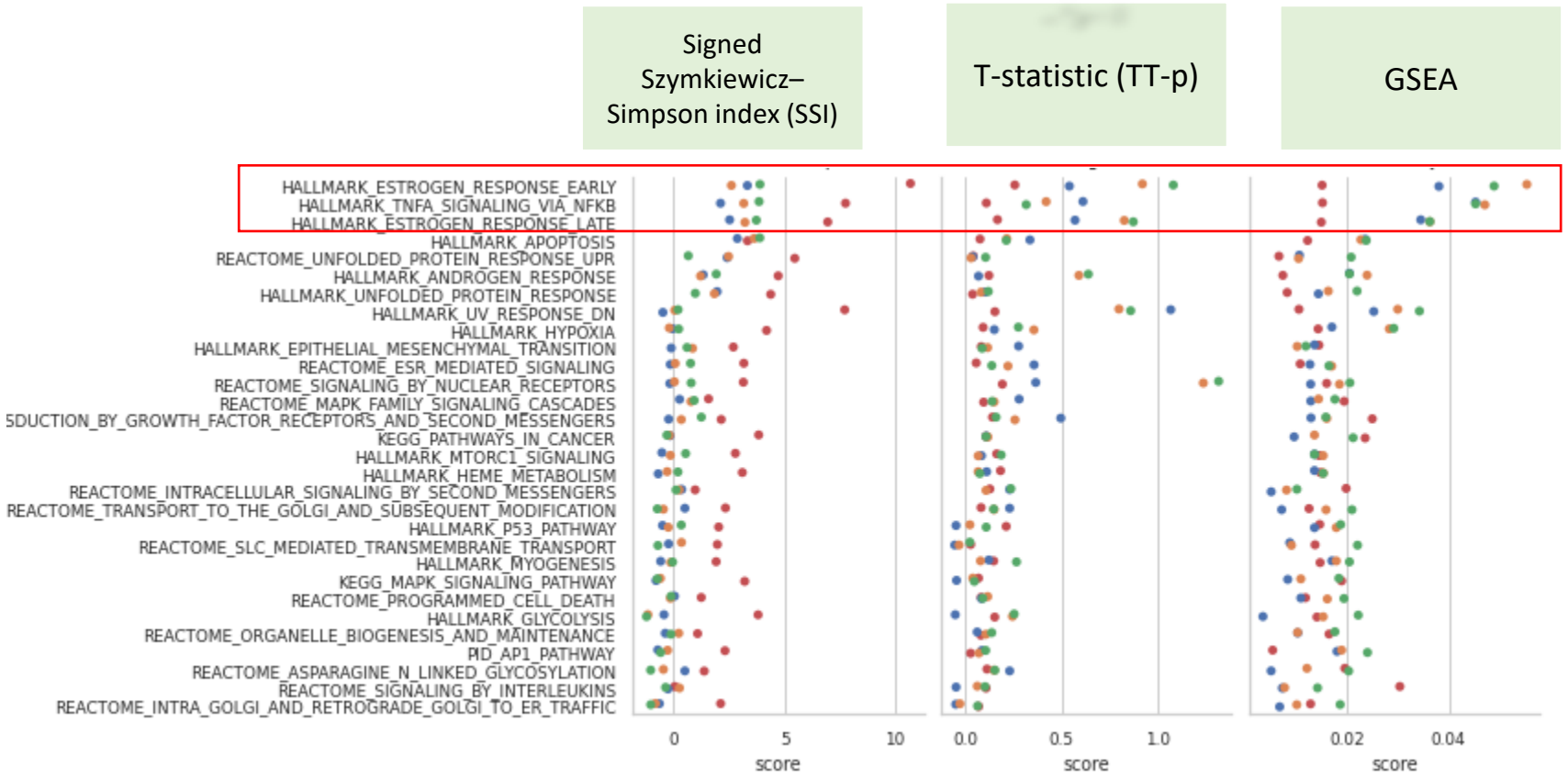
- Troglitazone is a thiazolidinedione (TZD) used as an antidiabetic and anti-inflammatory
- MIE: peroxisome proliferator activated receptor (PPARα) activator
- Use transcriptomic profile for troglitazone 10µM @ 6 h in PC3 cells
- Match 6,100 transcriptomic signatures (50-up and 50-dn genes) for 1200 chemicals in Connectivity Map v2
- Use three connectivity scores
- Best matches with other TZDs and PPARα-activators
- Can use this approach to identify putative PPARα activators





Matching estrogen transcriptomic profile with pathway signatures

- Estrogen is a female sex hormone activates the estrogen receptors (ERα/β)
- Use transcriptomic profile for troglitazone 10µM after 6 h in PC3 cells
- Match against 2,253 canonical and hallmark pathways in MSigDB v7.2
- Use different connectivity scoring methods and parameters
- Best matches are with estrogen response pathways
- Could use this approach to find putative ER-disruptors



Non-specific signatures: stress-response pathways

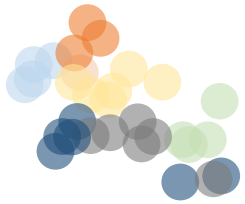
1

The Major Adaptive Stress response pathways

	Stress response pathway	Chemical inducers	TF	Activated gene promoters
OSR	Oxidative stress	Quinones, hydroperoxides, heavy metals, trivalent arsenicals	Nrf2	*HMOX1*, *NQO1*, *GST2A*
HSR	Heat shock response	Heat, Heavy Metals	HSF-1	*HSPA6*
DDR	DNA damage response	Etoposide, Methyl Methanesulfonate, N-Dimethylnitrosamine, Cyclophosphamide, UV radiation	p53	*CDKNIA*, *GADD45A*, *MDM2*, *BCL2*, *TP53I3*
HPX	Hypoxia	Hypoxia, Cobalt, Desferriozamine, Quercetin, Dimethyloxalylglycine	HIF-1	*VEGF*, *TF*, *EPO*
UPR	ER stress	Tunicamycin, Thapsigargin, Caplain, Brefeldin A	XBP-1, ATF6, ATF4	*HSP90B1*, *HSPA5*, *DNAJB9*
MTL	Metal stress	Heavy Metals	MTF-1	*MT1E*, *MT2A*
	Inflammation	Metal, PCBs, Exhaust Particles, Smoke Particles	NF-κB	*IL1A*, *TNFA*
	Osmotic stress	High salt, polyethylene glycol, mannitol	NFAT5	*AKR1B1*, *SLC6A12*, *SLC5A3*

Simmons et al., 2009

2



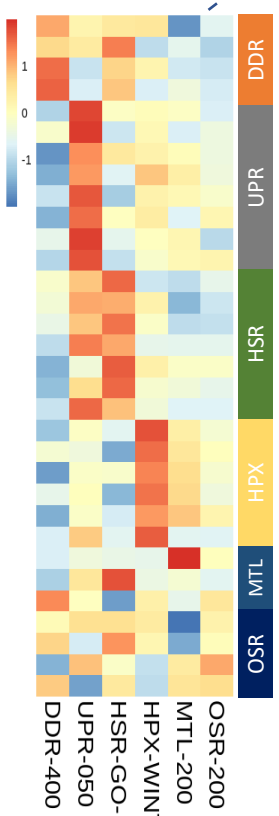
Published Signatures (MSigDB v7.2)

Overlapping Consensus Genes

Unique Consensus Signatures

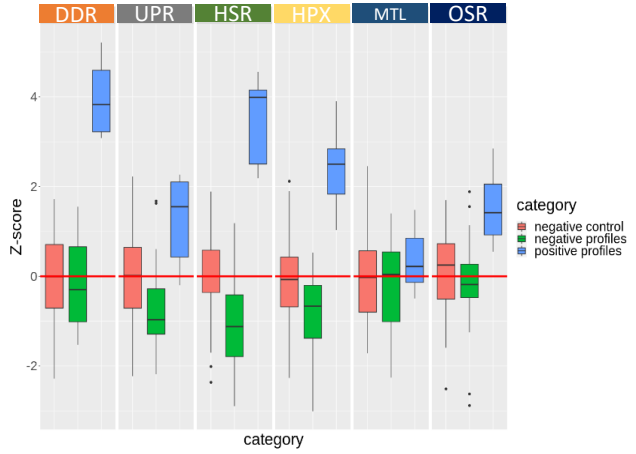
Connectivity Mapping with Transcriptomic Data for reference Chemicals (GSEA)

3



Performance

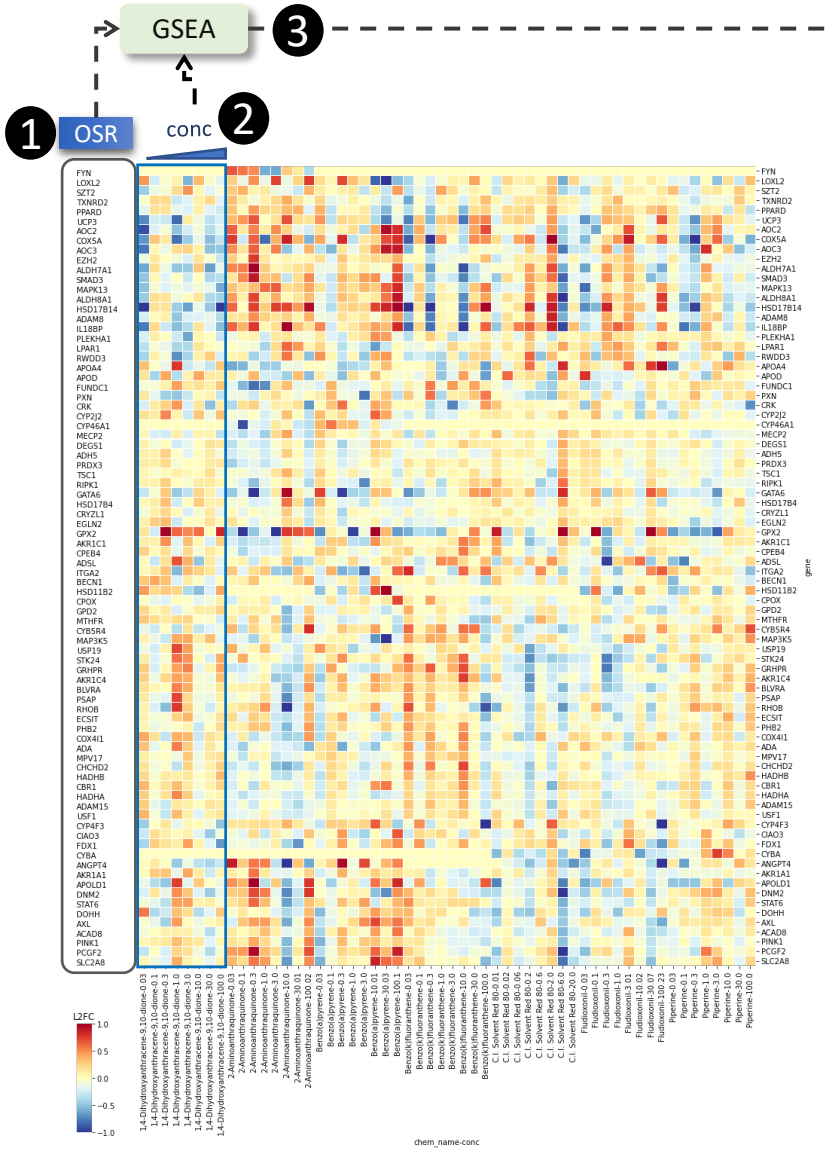
4

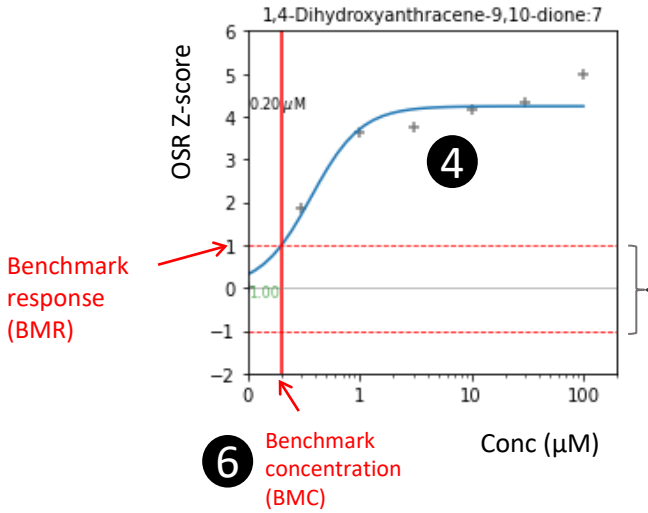


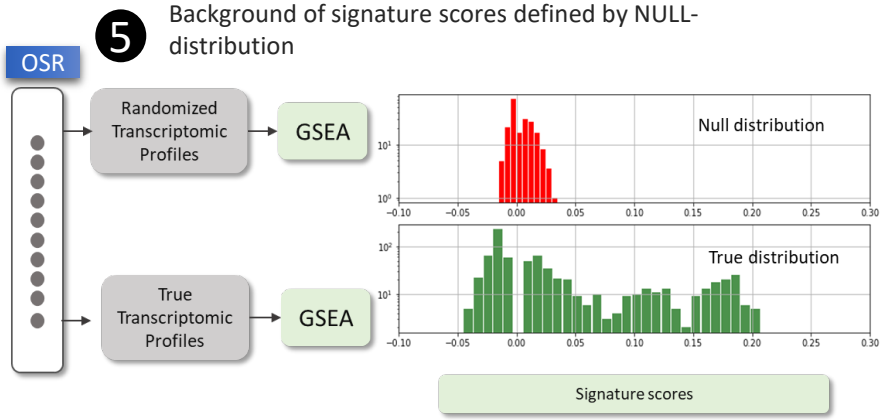
- Consensus signatures for most SRPs can identify reference perturbagens
- Can use SRP signatures to evaluate non-specific chemicals

Chambers *et al.* (*submitted*)

Using signature scores to estimate PODs

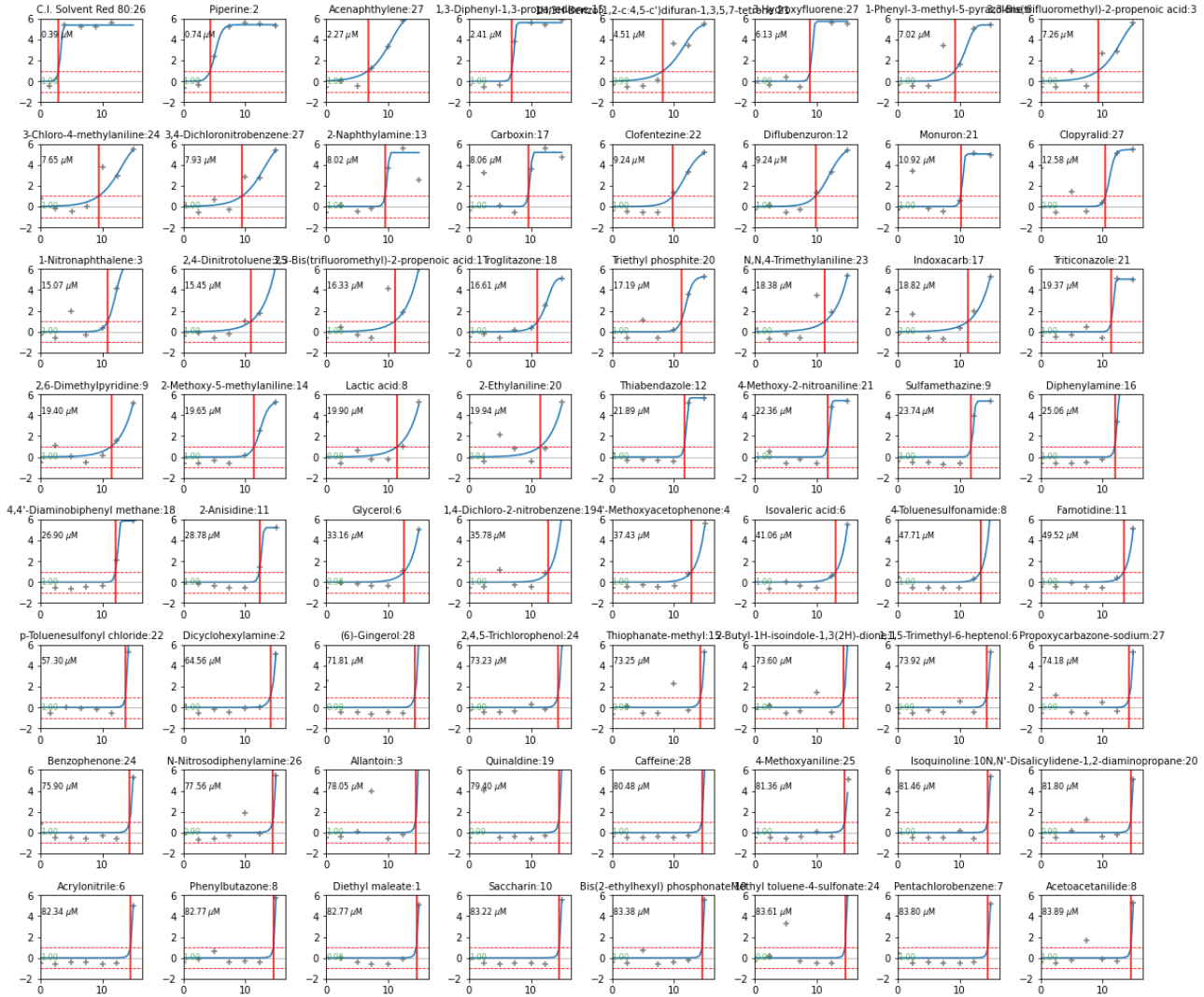


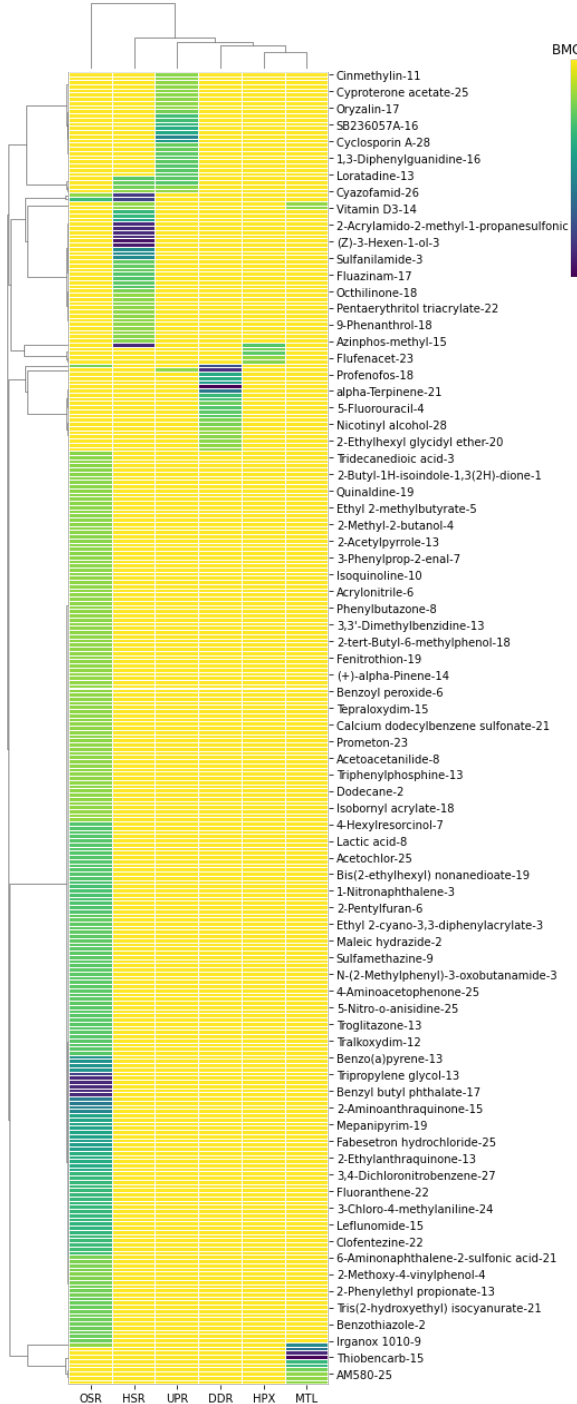




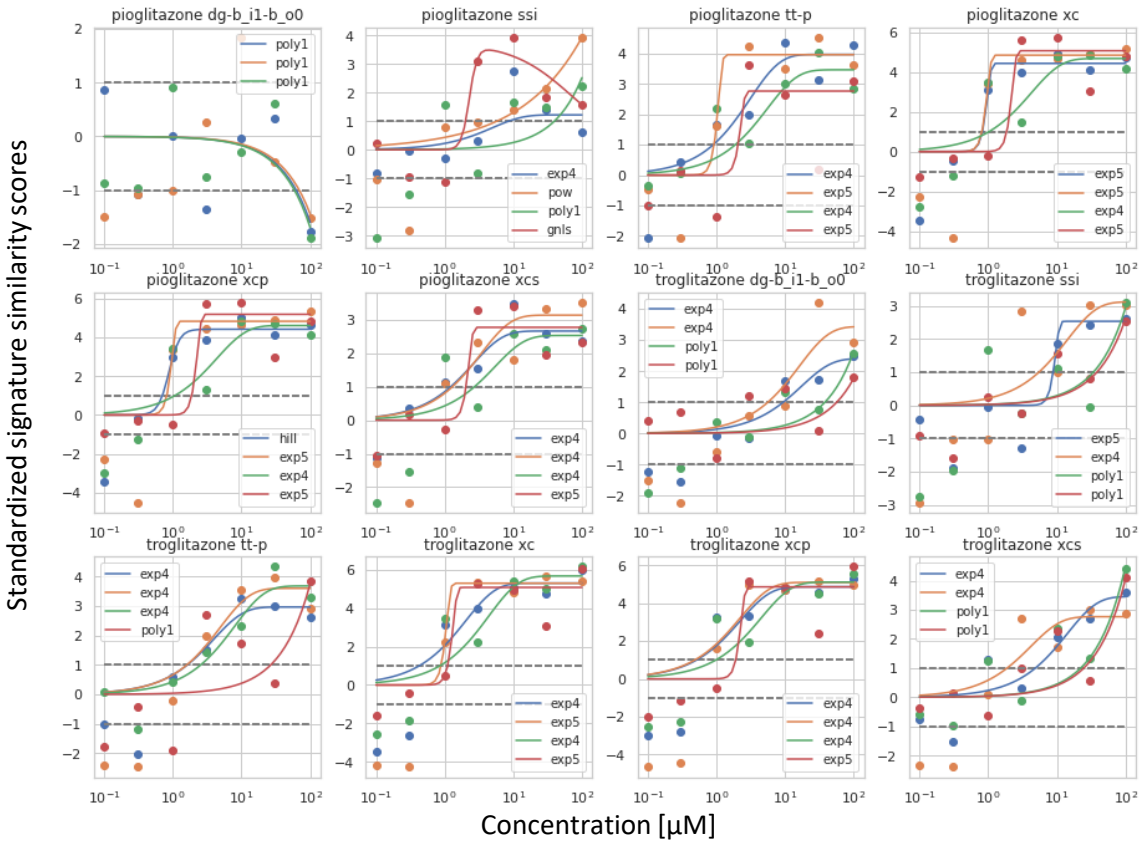
- Use
- Standardize signature scores using background distribution (Z)
- Concentration-response modeling using tcplFit2 (cnst, hill, gnls, poly1, poly2, pow, exp2, exp3, exp4, exp5)
- Estimate benchmark concentration (BMC) using benchmark response (BMR) of Z=1

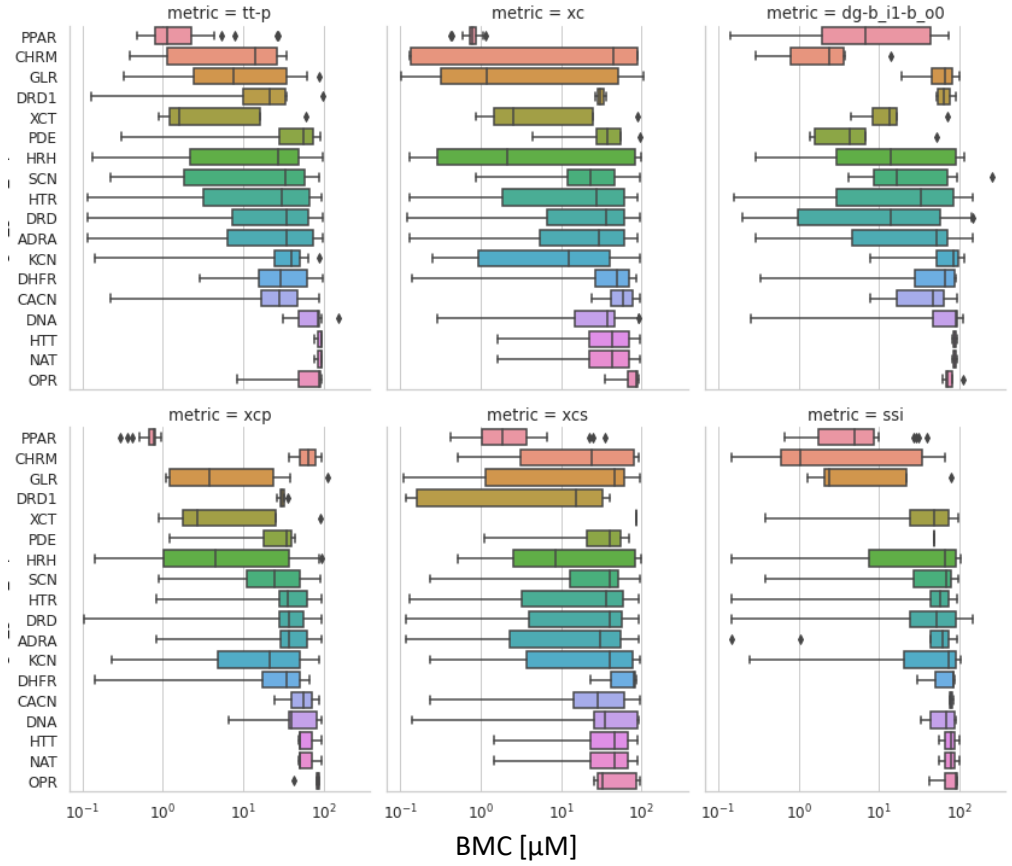
PODs for all chemicals and SRPs





Signature similarity scores to PODs: Troglitazone

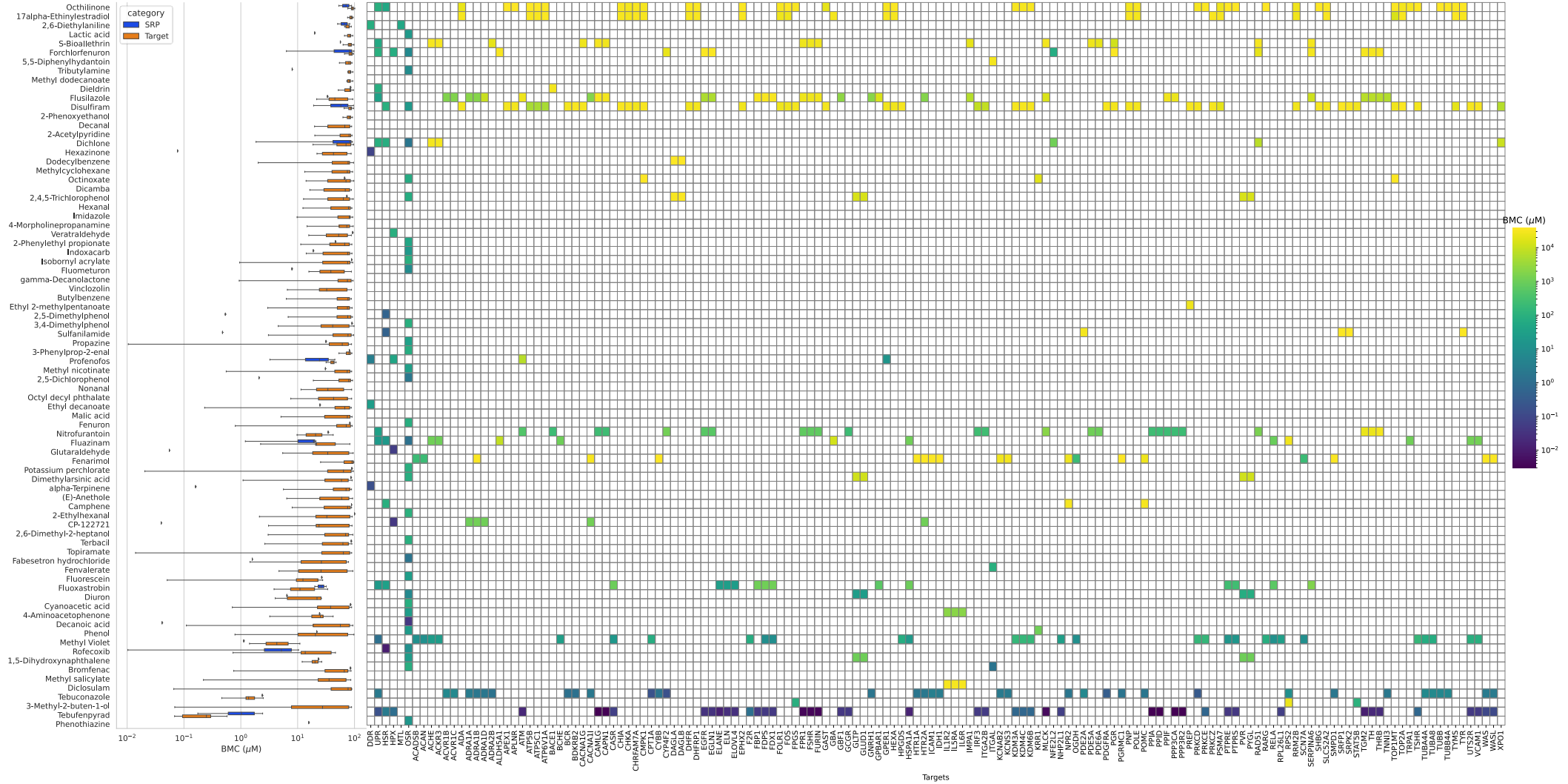




- Data: HepaRG cells treated with 1,366 chemicals 0.01-100μM
- Query: Troglitazone profiles replicated across 29 batches
- Reference: CMap v2 Affymetrix data
- Connectivity analysis using multiple signatures and metrics
- Score signatures against random profiles to estimate background

- Standardize similarity scores using background distribution (Z)
- Concentration-response modeling using tcplFit2 (cnst, hill, gnls, poly1, poly2, pow, exp2, exp3, exp4, exp5)
- Estimate benchmark concentration (BMC) using benchmark response (BMR) of Z=1

Summary of hits for HepaRG treatments



Summary

1. HTTr using TempO-Seq is a reliable & scalable tool for NAM development
2. Connectivity mapping uses *multivariate* gene signature scores for hazards and PODs
3. Additional evaluation of connectivity scoring & gene signatures is necessary
4. We are comparing PODs from transcriptomics with traditional values using qIVIVE
5. Adaptive stress response signatures is may help distinguishing specific vs. non-specific chemicals

Summary

1. HTTr using TempO-Seq is a reliable & scalable tool for NAM development

   We are using TempO-Seq technology to evaluate thousands of chemicals in multiple cell lines and have developed a high-throughput pipeline to process and analyze transcriptomic concentration-response data.

2. Connectivity mapping uses *multivariate* gene signature scores for hazards and PODs

   Environmental chemicals may not induce large changes in expression of individual genes. Gene signature-based connectivity scoring is more sensitive and can accurately identify putative targets.

3. Further evaluation of connectivity scoring & gene signatures

   We are systematically evaluating parameters of connectivity mapping and gene signature development to determine best practices for different chemical categories using gecco software.

4. Future directions

   - Systems biology of adaptive stress response pathways to distinguish specific vs non-specific chemicals
   - Investigate the molecular basis of cellular resilience and tipping points
   - Study single-cell analysis to further improve confidence in HTTr data

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