Rapid Toxicity Screening of Chemicals Combining *In Vitro* High-throughput Transcriptomics, Toxicokinetics and Exposure Estimates

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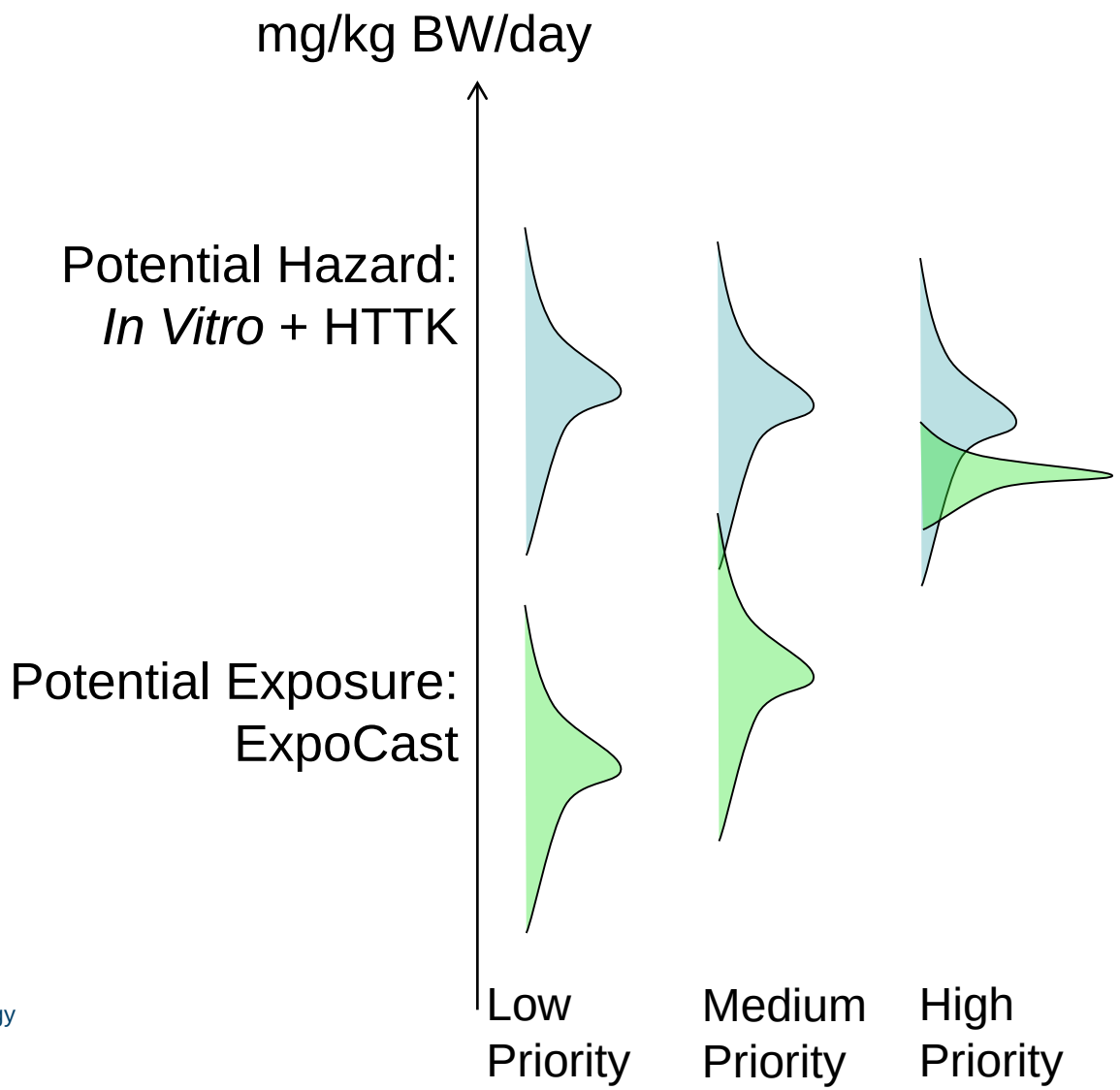
Themes

- Too many chemicals:
  - Can't test all chemicals in animal studies
  - Need alternative method (New Approach Methods / NAMs)
  - Goal: Replacement or Prioritization
- NAMs need to cover lots of biology
  - Many biological domains, require many individual *in vitro* assays or models (e.g. QSAR)
  - High-throughout whole genome transcriptomics is now practical
- Think about Risk
  - *In vitro* assays need to predict dose values (mg/kg/day)
  - Need to compare hazard with exposure

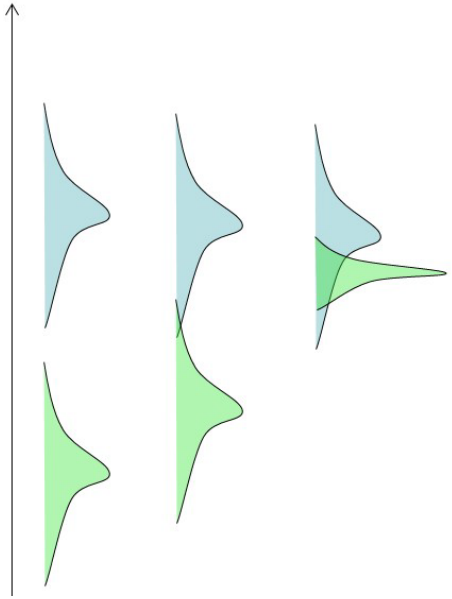
Risk-based Approach
Hazard + Exposure + Uncertainty

Semi-quantitative *In Vitro* to *In Vivo* Approach



Tools / Models / Data needed

- Hazard information or model
  - Start with *in vitro* data
  - Quantify concentration (μM) required to trigger bioactivity
- Toxicokinetics
  - Use to convert between external dose and internal concentration
- Exposure information or model
  - Quantify in mg/kg/day
- Include uncertainties everywhere

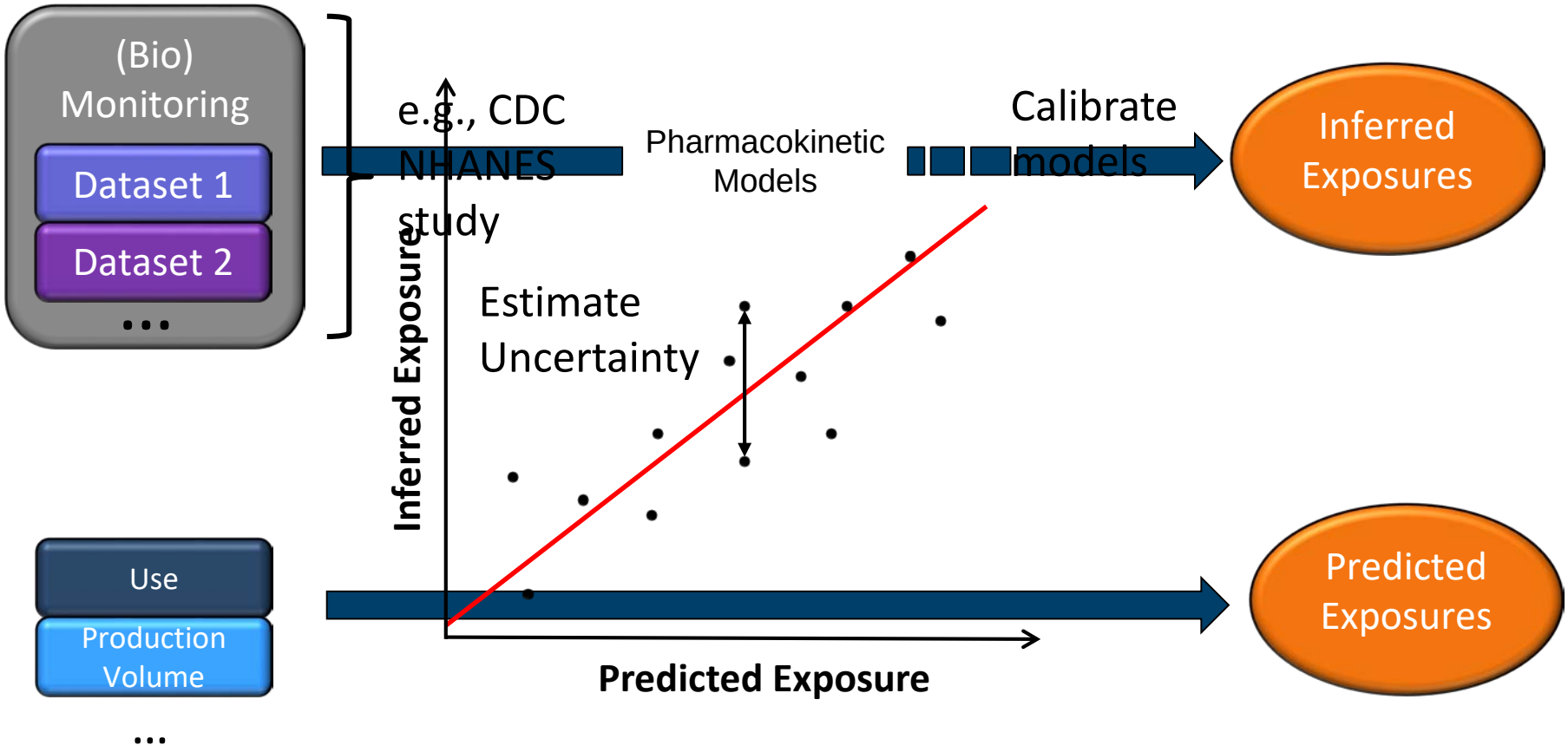


Hazard Approach where Animal Data is Lacking

- Goals:
  1. Quantitative point of departure (POD) (e.g. NOAEL)
  2. Estimate of what effects will be seen (e.g. liver hypertrophy)
- Experimental approaches
  - Battery of *in vitro* assays (ToxCast), one per target / pathway
  - High-throughput whole genome transcriptomics
  - Yield POD and MOA / AOP / mechanism information
- Modeling approaches
  - QSAR models
  - Read-across
  - TTC
  - Better at POD estimation than mechanism prediction

Population and Exposure Modeling

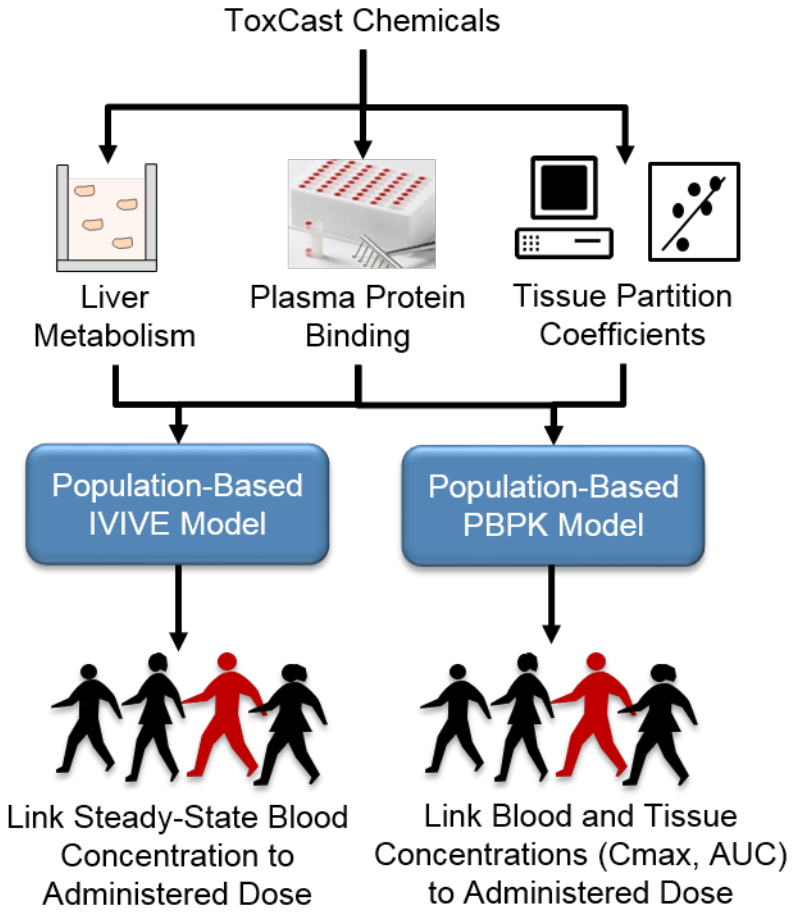
Estimating Exposure and Associated Uncertainty with Limited Data



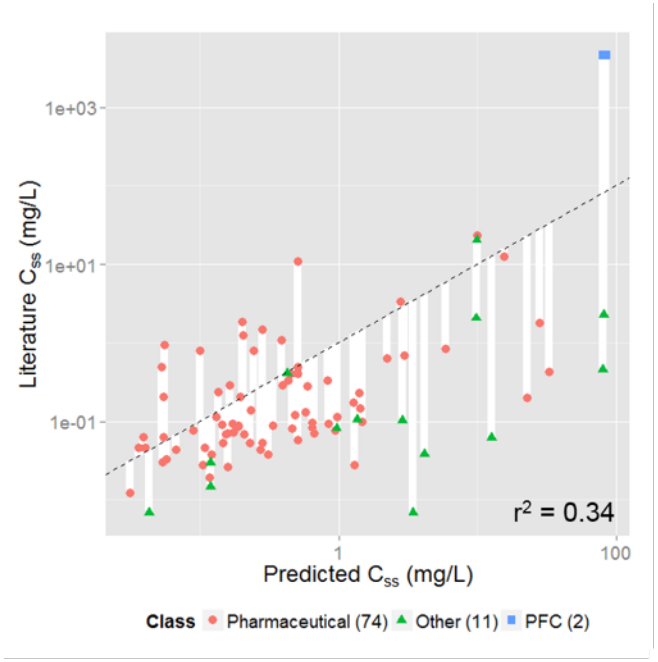
Wambaugh *et al.*, 2014

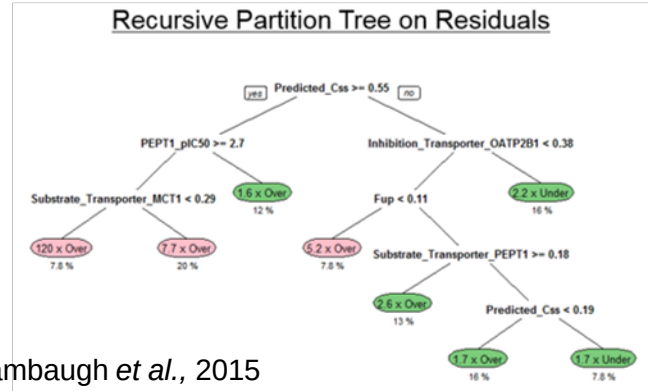
Toxicokinetics Modeling

Incorporating Dosimetry and Uncertainty into In Vitro Screening



Wetmore et al.





Wambaugh *et al.*, 2015

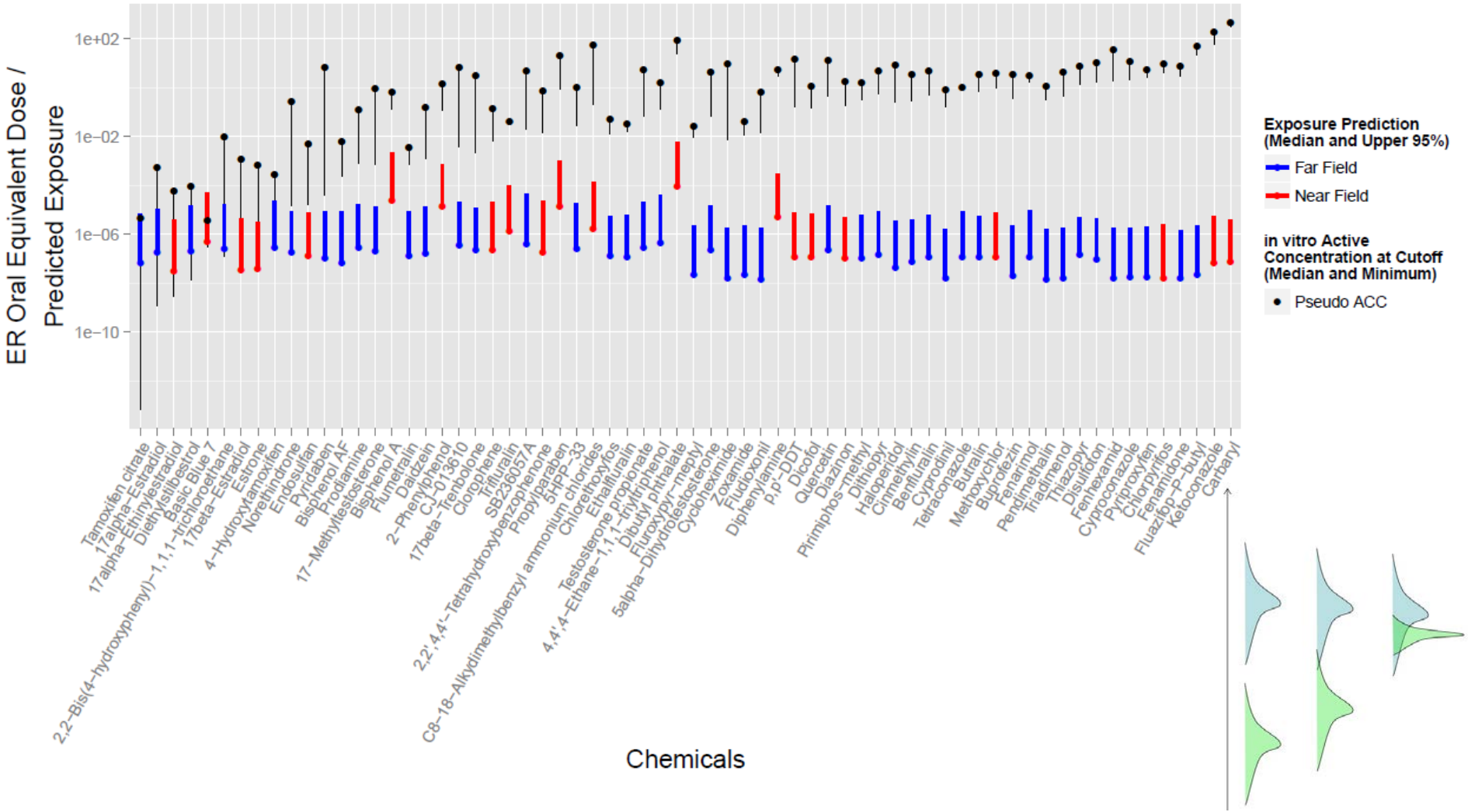
Putting it all together

- *In vitro* assays yield POD in μM
  - Select the minimum "relevant" *in vitro* POD
- TK yields *in vitro* to *in vivo* conversion factor
  - "Concentration at Steady State", C_{ss}
  - Blood concentration for a 1 mg/kg/day steady-state dose
- IVIVE POD ("oral equivalent dose") = *in vitro* POD / C_{ss}
- Exposure model yields estimate of exposure (mg/kg/day)

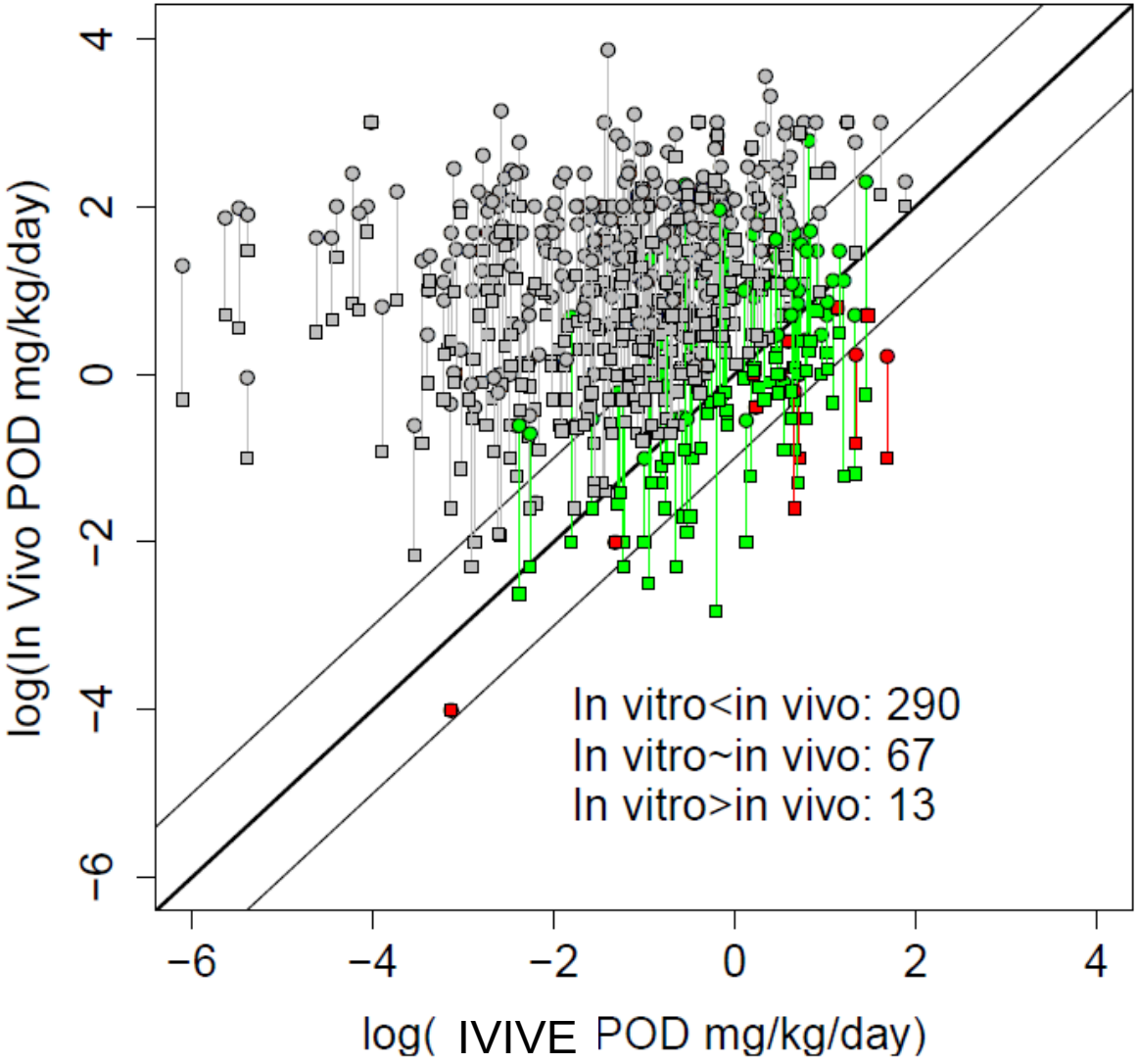
- BER: Bioactivity to Exposure Ratio
  - IVIVE POD / Exposure estimate
  - BER >> 1 implies low concern for risk

Prioritization (Replacement) Example
Compare predicted exposure and hazard POD

Compare estrogen receptor assay battery and exposure model



IVIVE PODs tend to provide low (protective) POD estimates: BERs are conservative



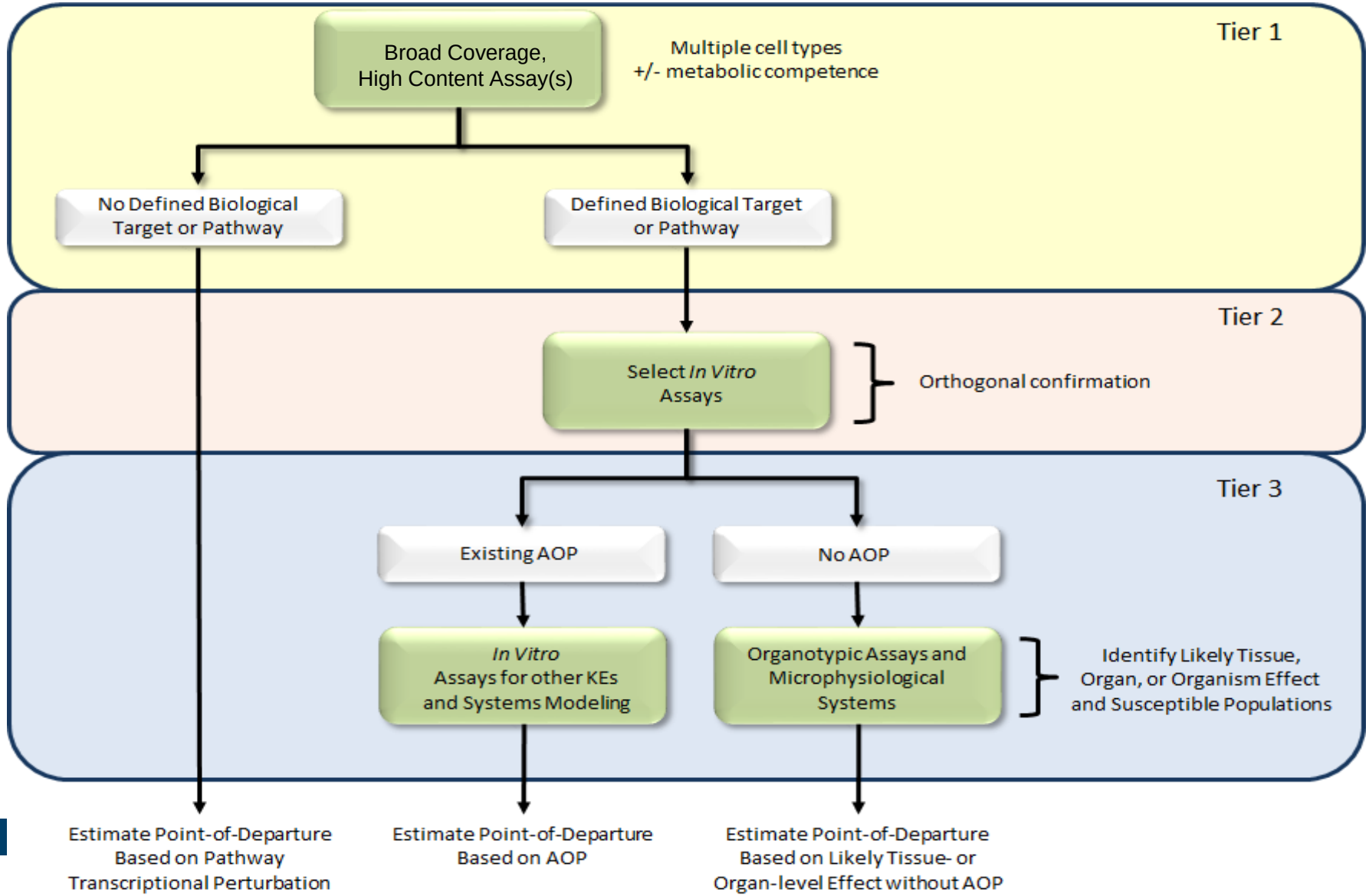
Only ~4% have *in vitro* POD consistently greater than *in vivo* values

Issue: what is the correct *in vitro* POD assay?

- Bioactivity vs. adversity

Work in progress: comparison of results taking into account both *in vivo* and *in vitro* uncertainties

Adding a Transcriptomics Front End

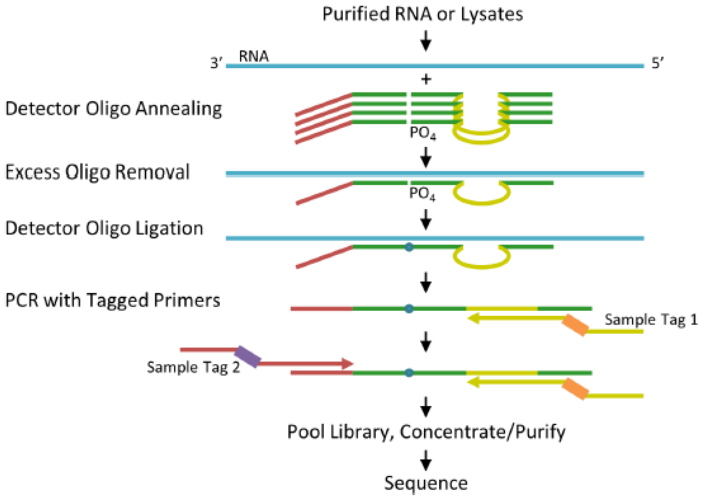


BioSpyder TempO-Seq Technology Overview

Technology

- 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**.
- Transcripts in cell lysates generated in 384-well format are barcoded according to well position and combined in a single library for sequencing using industry standard instrumentation.
- Scalable, targeted assay:
  - 1) Measures transcripts of interest
  - 2) Greater throughput and requires lower read depth than RNA-Seq
  - 3) Ability to attenuate highly expressed genes
- Per sample fastq files are generated and aligned to BioSpyder sequence manifest to generate integer count tables.

TempO-Seq Assay Illustration



Experimental Workflow

Cryopreserved Cell Stocks

Cell Expansion

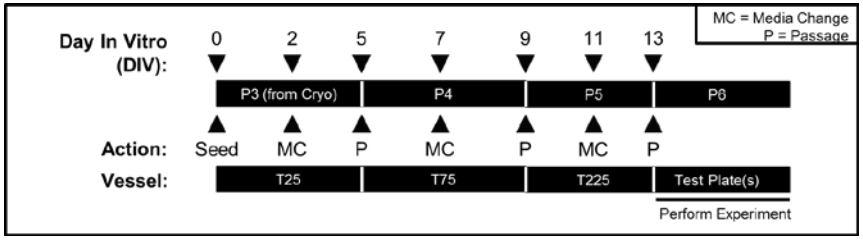
Cell Plating

BioTek MultiFlo ™ FX

Dispensing Test Chemicals

LabCyte Echo® 550 Liquid Handler

Standardized Expansion Protocol



Track 1: Targeted RNA-Seq

Generating Cell Lysates

TempO-Seq WT

Track 2: Apoptosis / Cell Viability

Reagent Dispensing

High Content Imaging

Perkin Elmer Opera Phenix™ High Content Screening System

Josh Harrill

HTTr MCF-7 Screen: Experimental Design

Parameter	Multiplier	Notes
Cell Type(s)	1	MCF-7 (ATCC® HTB-22™)
Culture Condition	1	DMEM + 10% HI-FBS
Chemicals	2,112	ToxCast ph1, ph2 Nominated chemicals from e1k / ph3
Time Points:	1	6 hours
Assay Formats:	2	TempO-Seq HCI Cell Viability & Apoptosis
Concentrations:	8	3.5 $\log_{10}$ units; semi $\log_{10}$ spacing
Biological Replicates:	3	--

- **Total number of samples:** 54,432
- **Total number of endpoint readouts:** 1.15×10^9
- **Total size of fastq files:** 32.5 TB

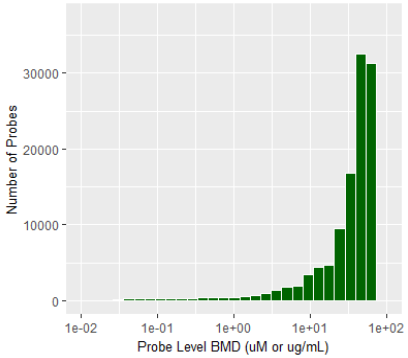
[a] MCF-7 cells cultured in DMEM + 10% HI-FBS was selected as the test system to facilitate comparability to the Broad Institute Connectivity Map (CMAP) database (http://portals.broadinstitute.org/cmap/).

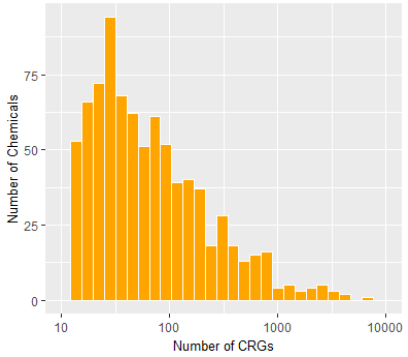
Analysis Approaches

- Gene level vs. Pathway Level
- Concentration-response modeling
- Different modeling approaches
  - Count-level: BMD Express, in-house methods
  - Log2 fold-change level: ToxCast Pipeline
- Statistical issues being investigated

Benchmark Dose Modeling Summary & Inducible Genes - BMDExpress

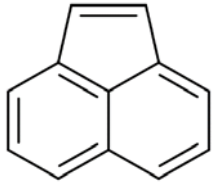
Expected genes show clean concentration-response

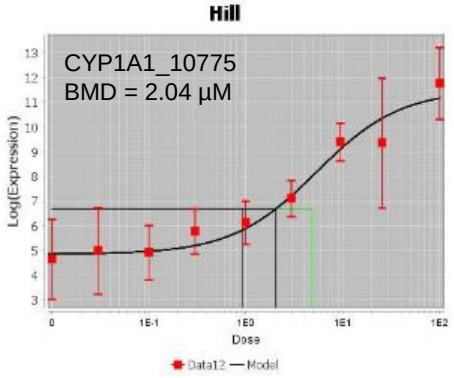




CYP1A1_10775
(n = 473)

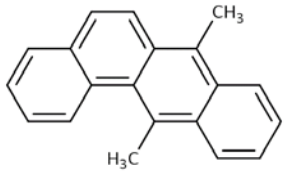
Acenaphthylene
208-96-8 | DTXSID3023845

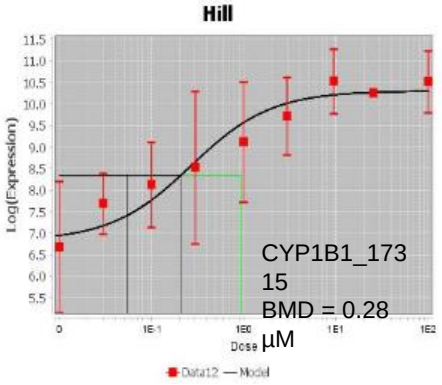




CYP1B1_17315
(n = 279)

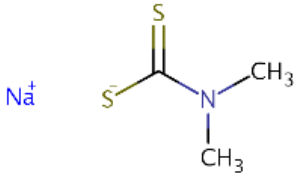
7,12-Dimethylbenz(a)anthracene
57-97-6 | DTXSID1020510

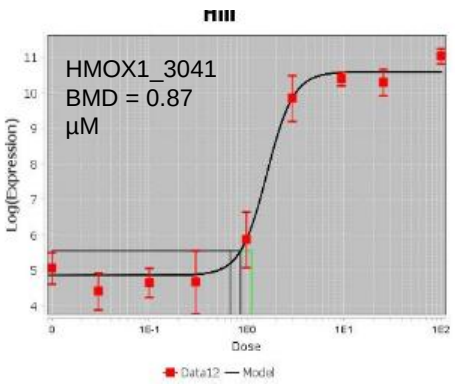




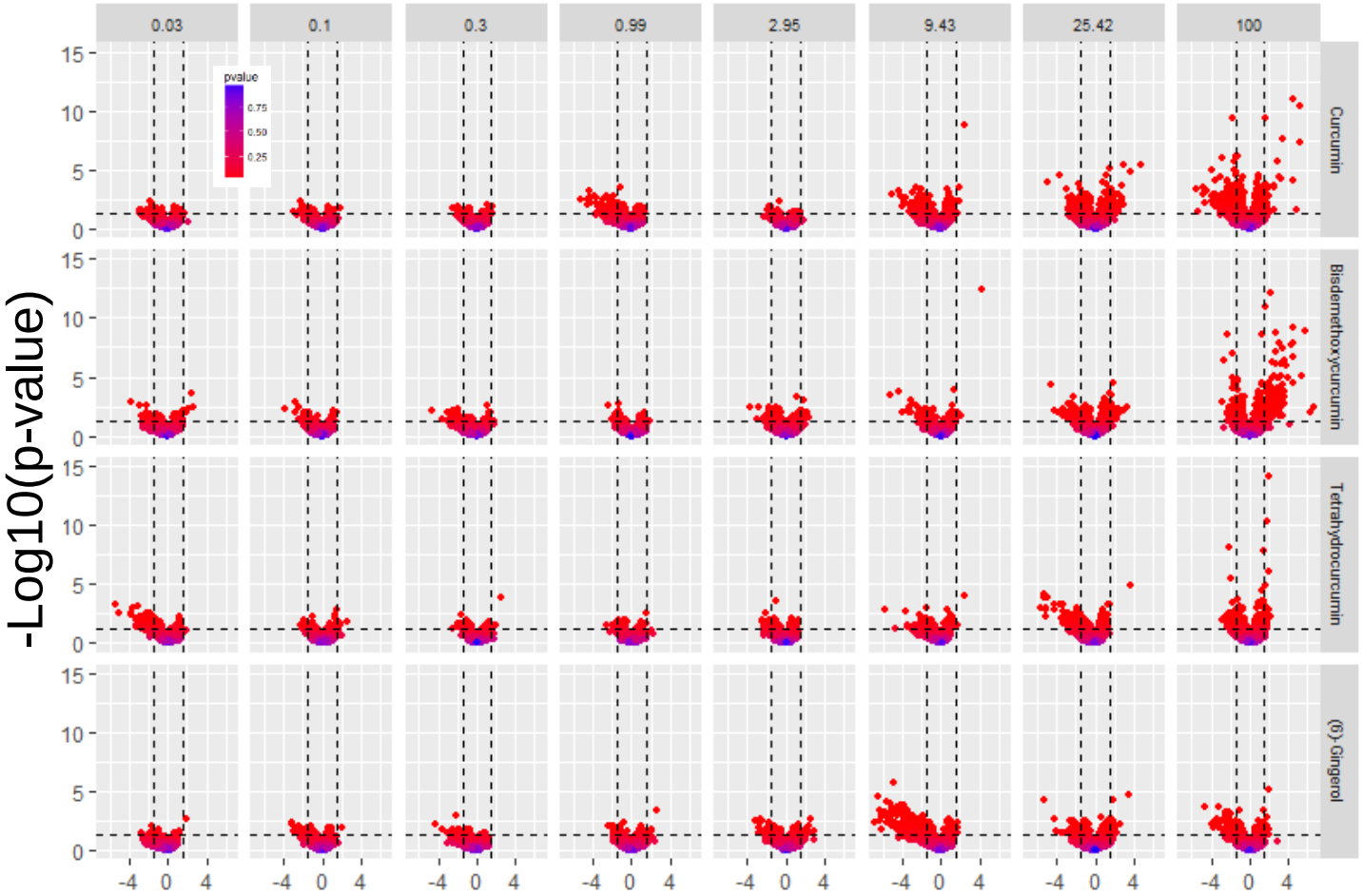
HMOX1_3041
(n = 174)

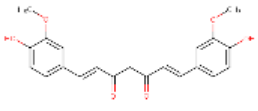
Sodium dimethyldithiocarbamate
128-04-1 | DTXSID6027050



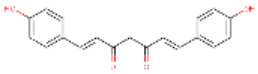


Evaluating Structurally Related Chemicals at the Multi-Gene Level

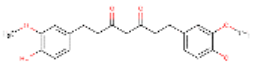




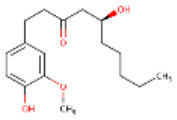
Curcumin | DTXSID8031077



Bisdemethoxycurcumin | DTXSID00872663

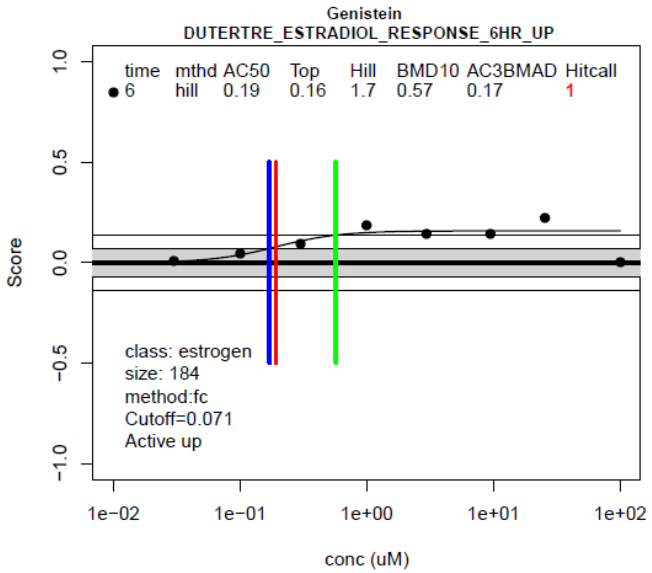


Tetrahydrocurcumin | DTXSID30865801

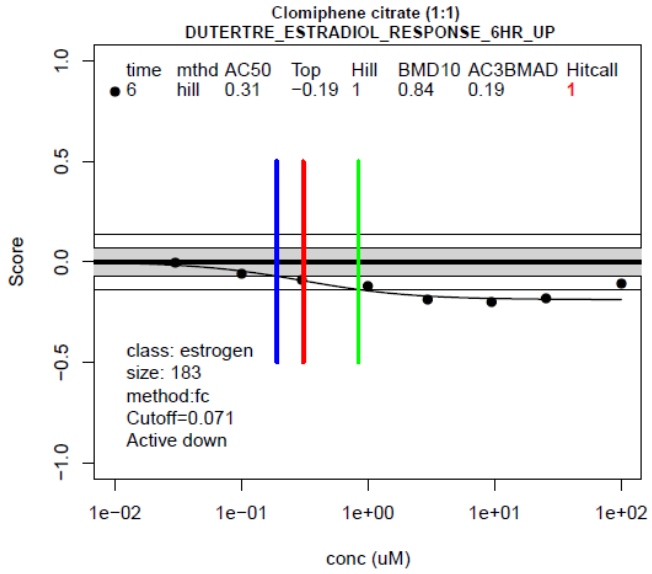


(6)-Gingerol | DTXSID3041035

Specific Pathway Concentration-Response (6-hour data)



Agonist



Antagonist

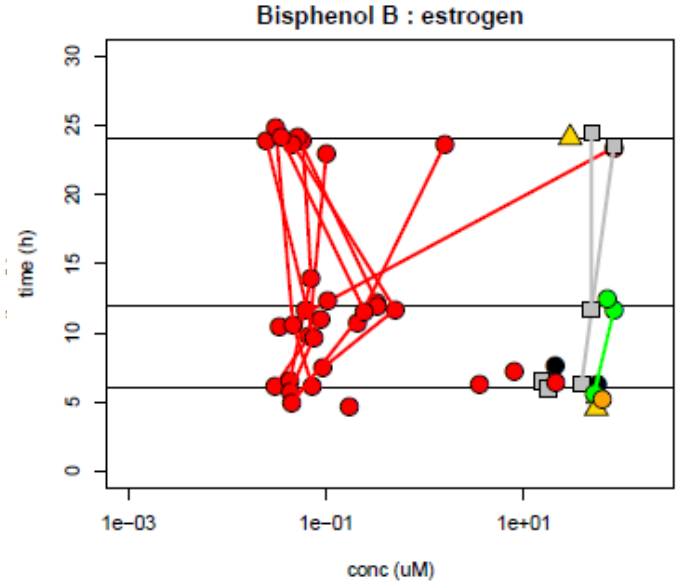
Example is an estrogen-receptor responsive pathway

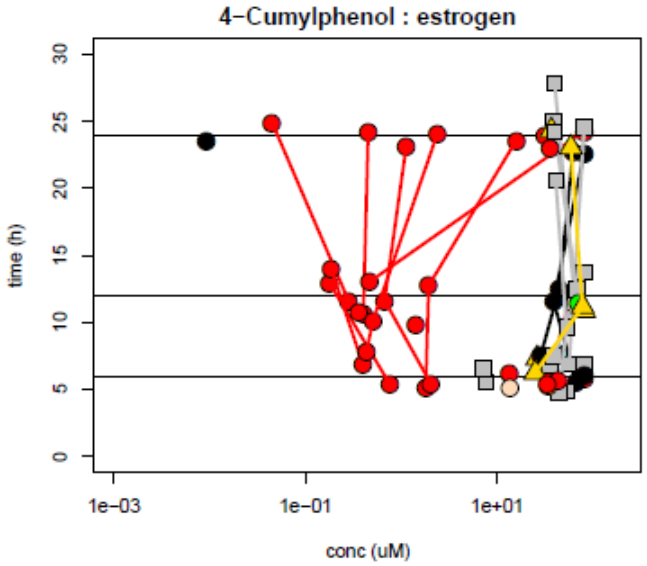
Relevant for MCF7 cells

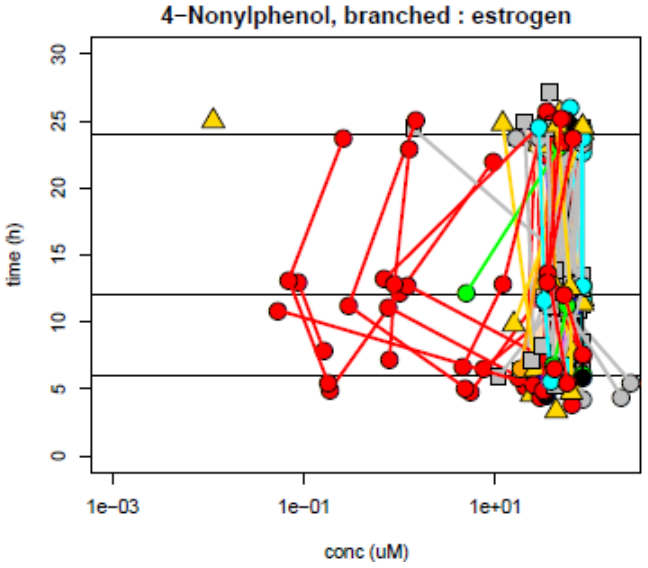
Individual genes have only small fold-change

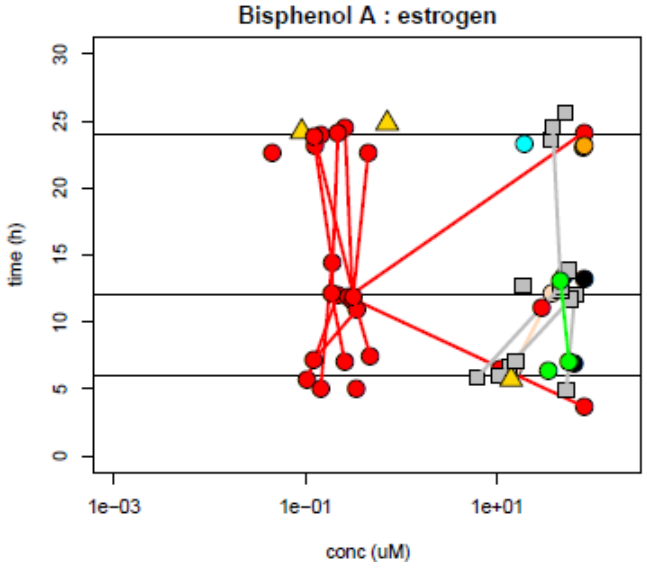
Pathway integration allows for signal to be seen

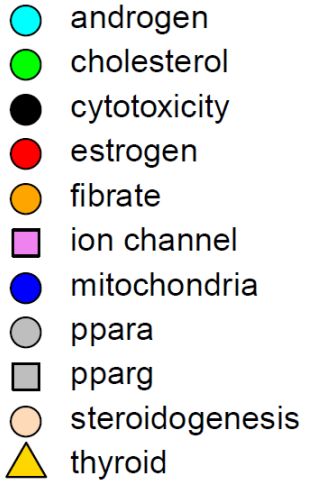
Pathway results across multiple times
Multiple estrogen pathways





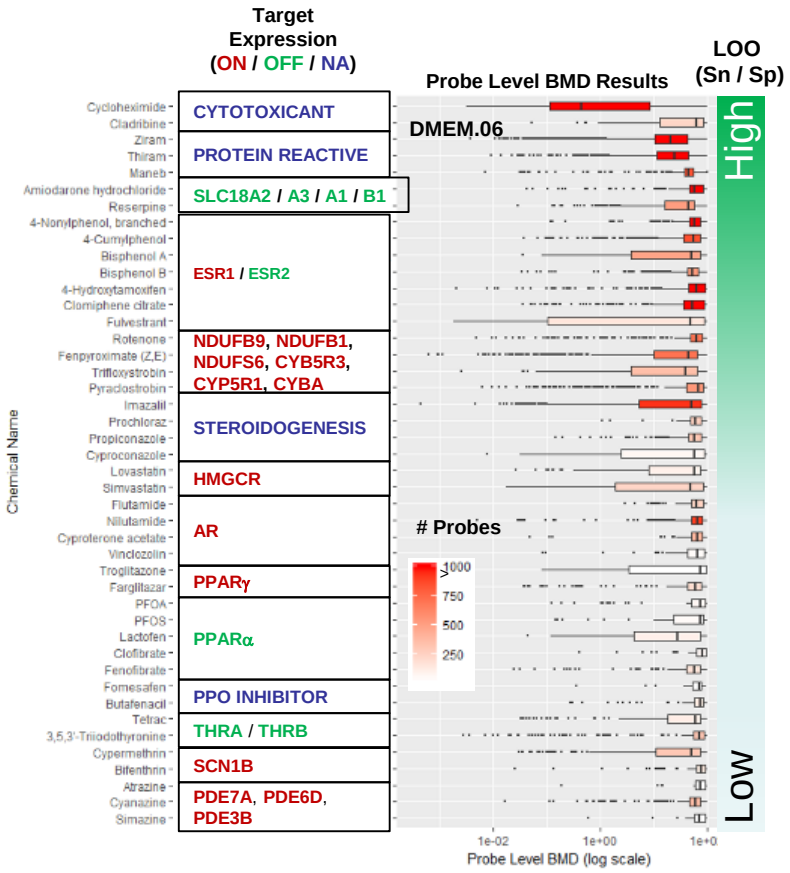


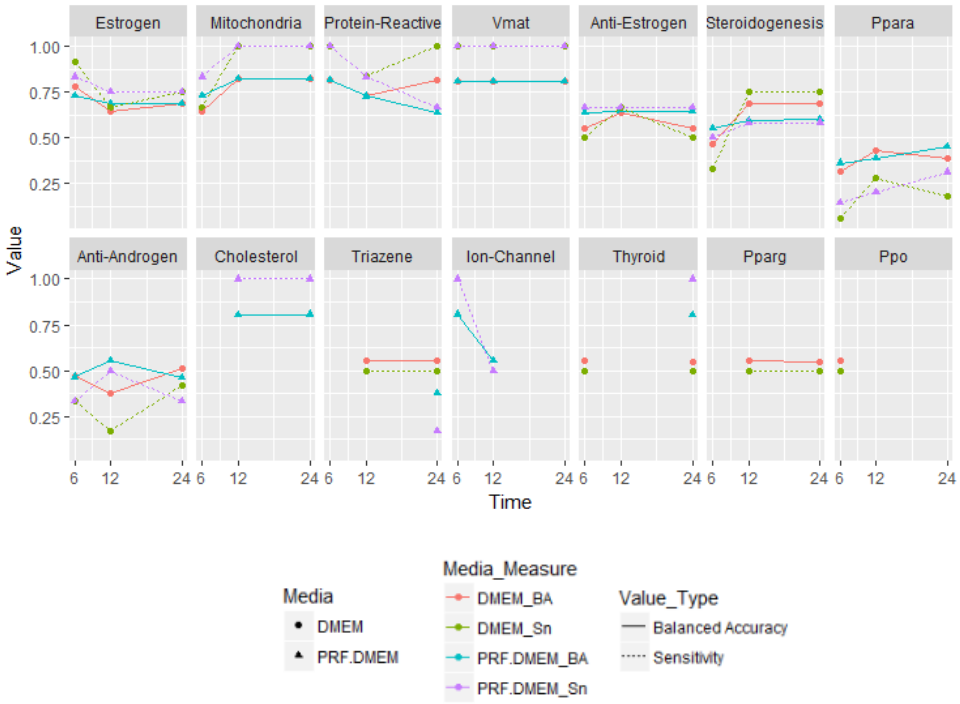




Points are AC50 values for pathway response

Connectivity Mapping for MoA Prediction





Summary

- Have overall process for predicting BER
  - Bioactivity (Toxicity) to Exposure Ratio (like margin of exposure)
  - Using in vitro (ToxCast) and modeled input
  - Can run on thousands of chemicals
  - Limitation: ToxCast covers a small part of biological space

- High-throughput transcriptomics has potential advantages over ToxCast
  - Larger biological space (10000 genes vs. 300)
  - No special cell engineering, can run on any cell type
  - Amenable to adding metabolic competence (see Steve Simmons talk)
  - Limitation: not complete, still need functional readout assays

Acknowledgments

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