High-Throughput Phenotypic Profiling with the Cell Painting Assay for Chemical Hazard Assessment and Determining Mechanisms of Action

Joshua A. Harrill
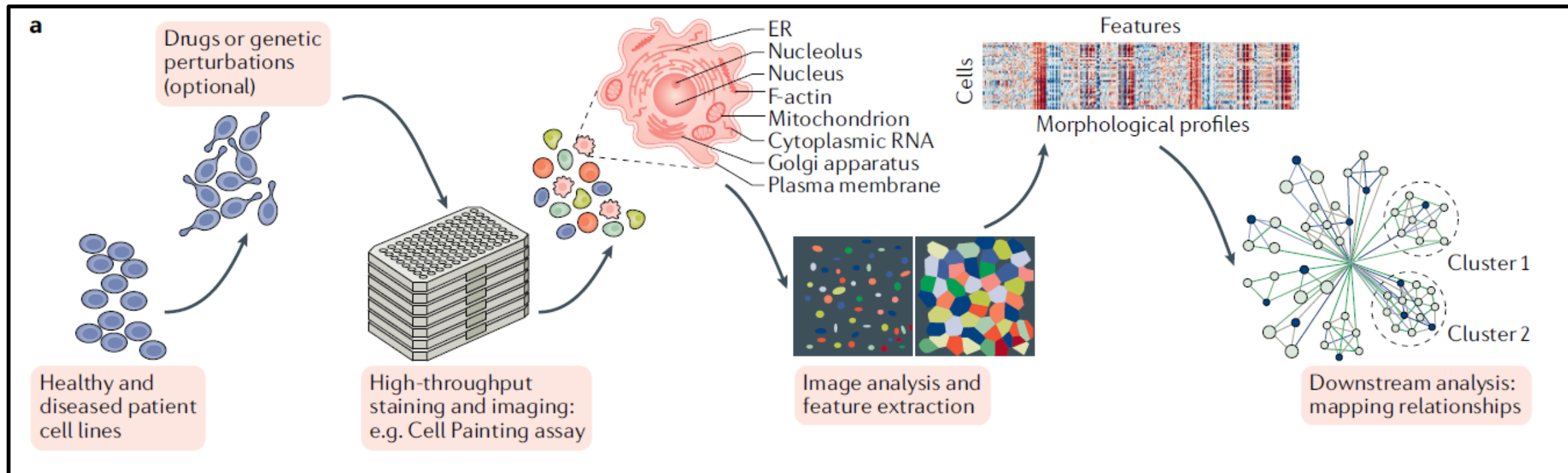
USEPA Center for Computational Toxicology and Exposure (CCTE)

Disclaimer

Imaging-Based High-Throughput Phenotypic Profiling (HTPP)

Chandrasekaran et al. Nat Rev Drug Discov. 2020 Dec 22:1–15

- A high-throughput testing strategy where rich information present in biological images is reduced to multidimensional numeric profiles and mined for information characteristic to a chemical's biological activity.

- Originated in the pharmaceutical sector and has been used in drug development to understand disease mechanisms and predict chemical activity, toxicity and/or mechanism-of-action.
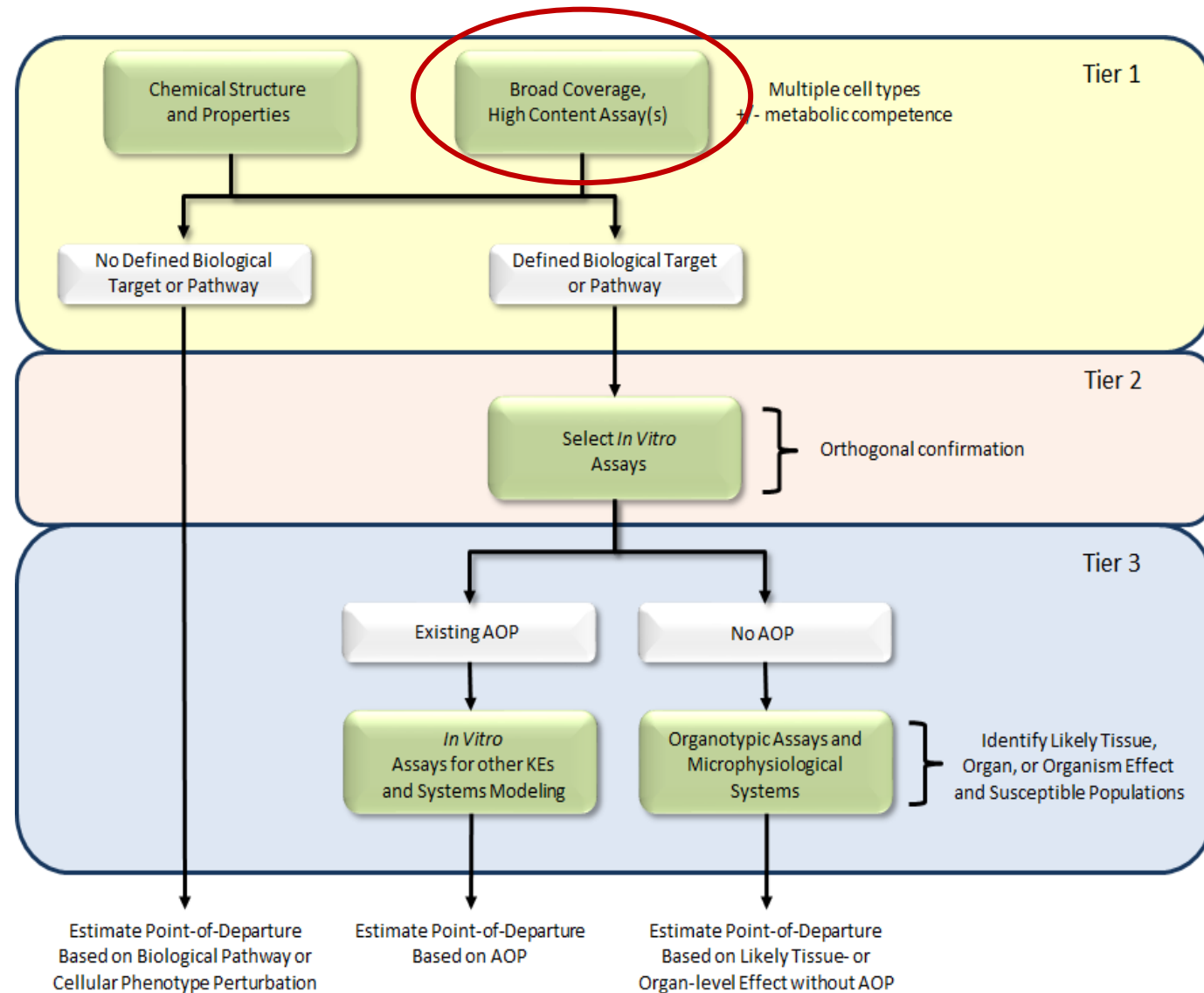
NAMs-Based Tiered Hazard Evaluation Approach

High throughput profiling (HTP) assays are proposed as the first tier in a NAMs-based hazard evaluation approach.

HTP Assay Criteria:
1. Yield bioactivity profiles that can be used for **potency estimation, mechanistic prediction** and evaluation of **chemical similarity**.
2. Compatible with multiple human-derived culture models.
3. Concentration-response screening mode.
4. Cost-effective.

To date, EPA has identified and implemented two HTP assays that meet this criteria.

- High-Throughput Transcriptomics [HTTr]
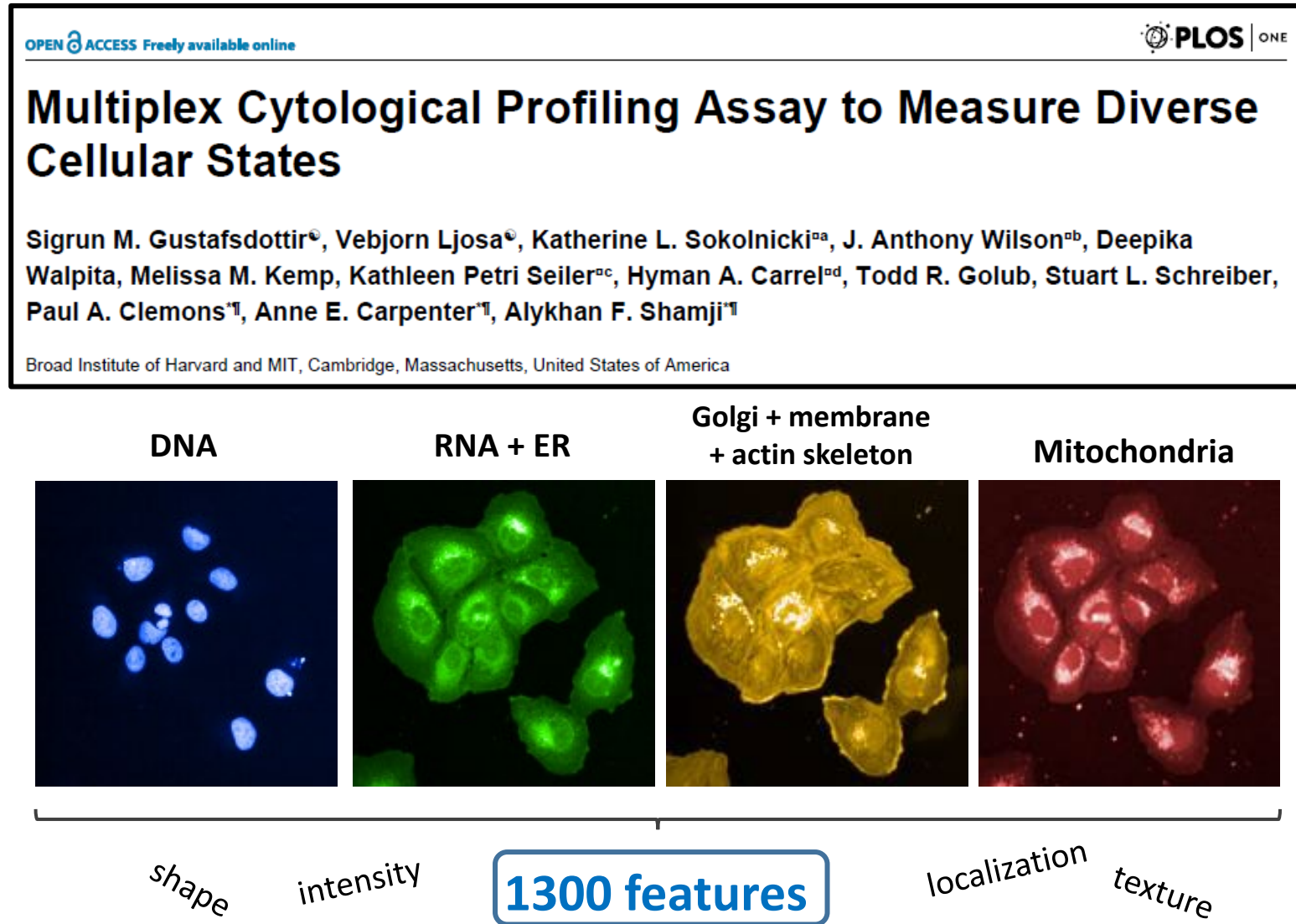- **High-Throughput Phenotypic Profiling [HTPP]**

Tier 1

Chemical Structure and Properties

Broad Coverage, High Content Assay(s)   Multiple cell types +- metabolic competence

No Defined Biological Target or Pathway

Defined Biological Target or Pathway

Tier 2

Select *In Vitro* Assays   Orthogonal confirmation

Tier 3

Existing AOP

No AOP

In Vitro Assays for other KEs and Systems Modeling

Organotypic Assays and Microphysiological Systems   Identify Likely Tissue, Organ, or Organism Effect and Susceptible Populations

Estimate Point-of-Departure Based on Biological Pathway or Cellular Phenotype Perturbation

Estimate Point-of-Departure Based on AOP

Estimate Point-of-Departure Based on Likely Tissue- or Organ-level Effect without AOP

The NexGen Blueprint of Computational Toxicology at US EPA
Thomas et al. (2019) DOI: 10.1093/toxsci/kfz058

HTPP with the Cell Painting Assay

Cell Painting is a profiling method that measures a large variety of phenotypic features in fluoroprobe labeled cells *in vitro*.

- High-throughput

- Scalable

- Amenable to lab automation

- Deployable across multiple human-derived cell types.

- Reproducible

- Cost-effective (¢ / well)

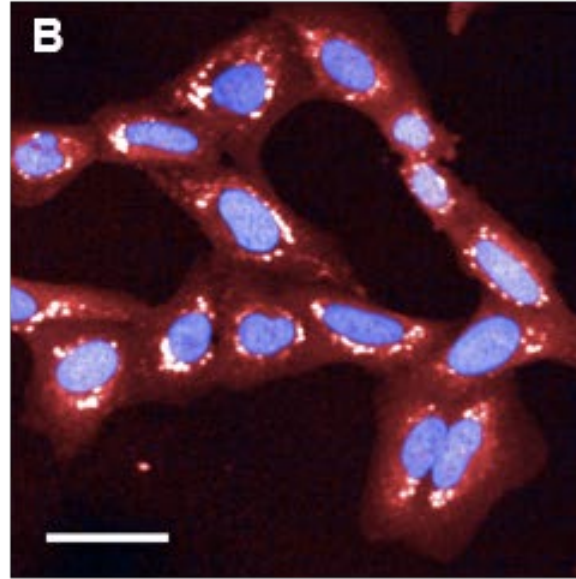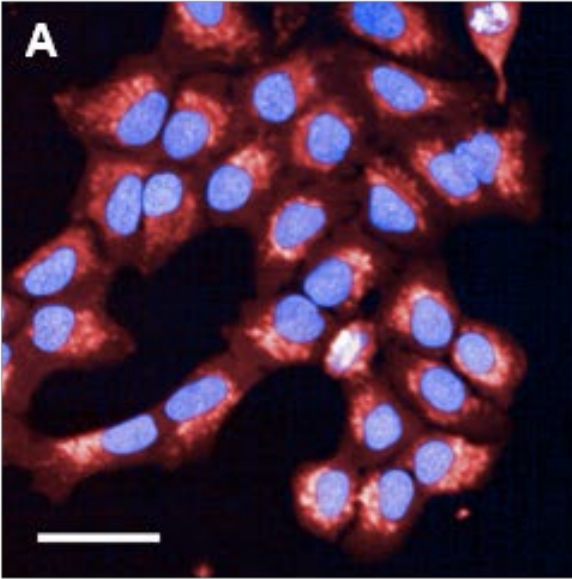- Infrastructure investment

- High volume data management

Laboratory & bioinformatics workflows for conduct of this assay have been established at EPA.

OPEN ⊚ ACCESS Freely available online   ⊚ PLOS | ONE

Multiplex Cytological Profiling Assay to Measure Diverse Cellular States

Sigrun M. Gustafsdottir©, Vebjorn Ljosa©, Katherine L. Sokolnicki[a], J. Anthony Wilson[b], Deepika Walpita, Melissa M. Kemp, Kathleen Petri Seiler[c], Hyman A. Carrel[d], Todd R. Golub, Stuart L. Schreiber, Paul A. Clemons[*¶], Anne E. Carpenter[*¶], Alykhan F. Shamji[*¶]

Broad Institute of Harvard and MIT, Cambridge, Massachusetts, United States of America

| DNA | RNA + ER | Golgi + membrane + actin skeleton | Mitochondria |

shape   intensity   **1300 features**   localization   texture
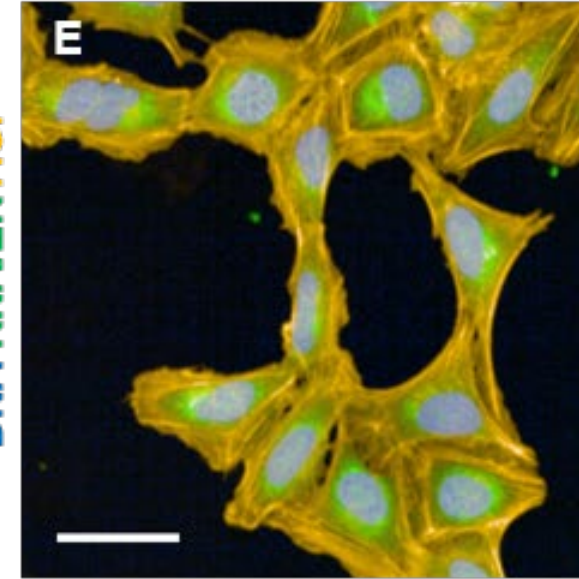
Example Chemicals

Solvent control (0.5% DMSO)   Berberine chloride (10 μM)

→ Mitochondrial compactness/texture
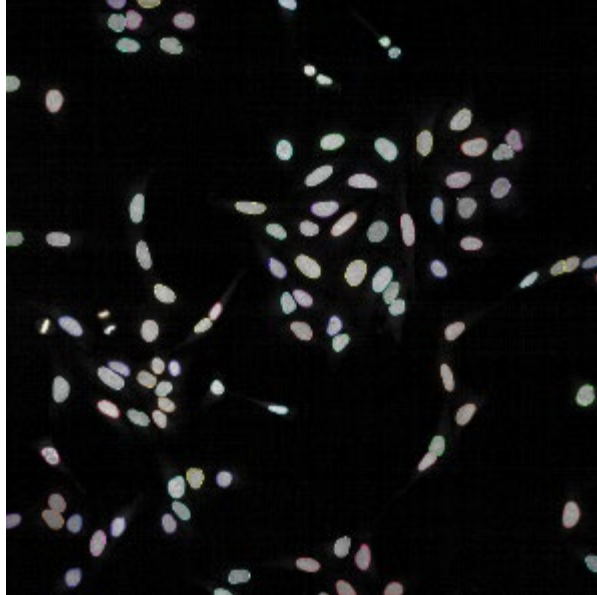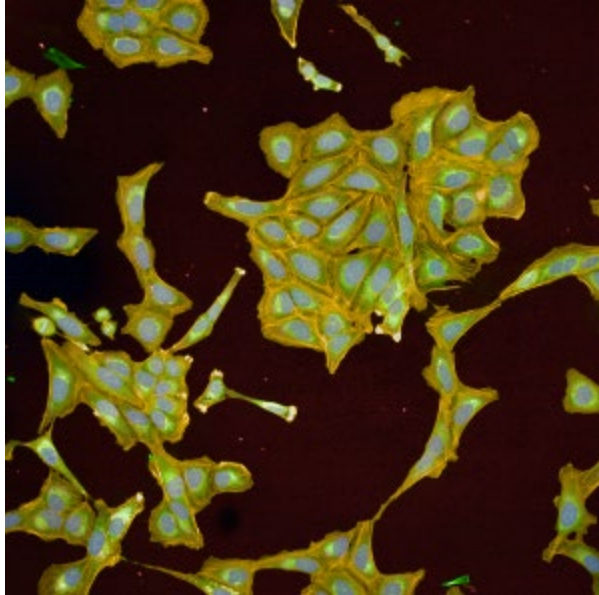
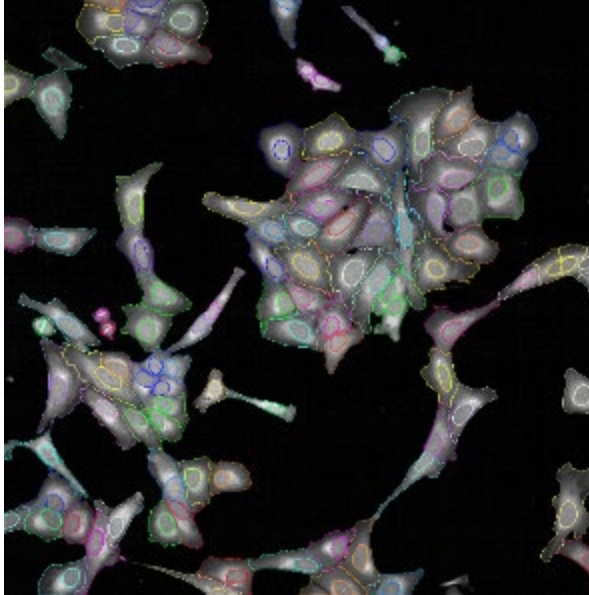Solvent control (0.5% DMSO)   Etoposide (3 μM)
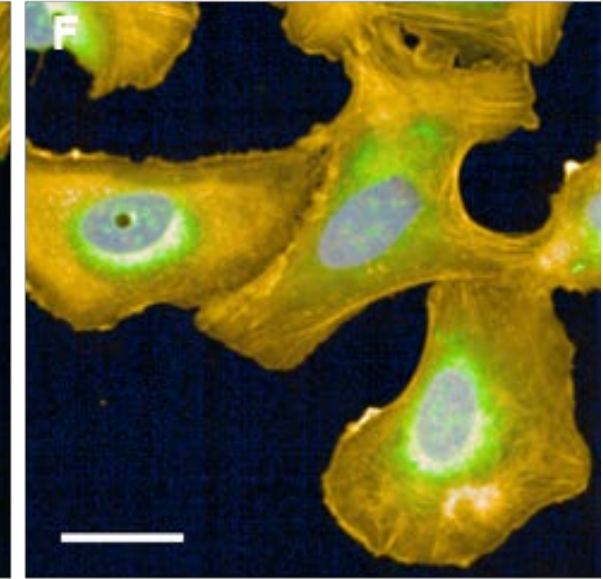
→ Cells are larger

- Strong phenotypes are observable qualitatively and can be measured quantitatively using Cell Painting
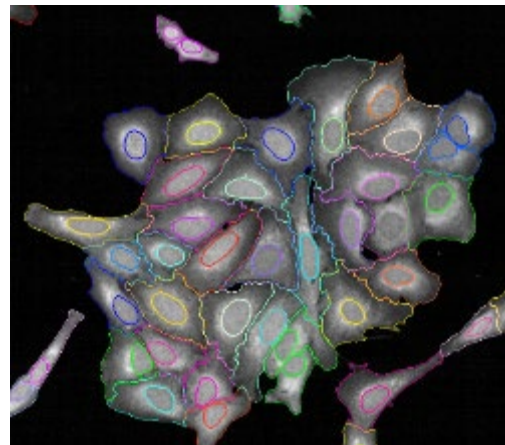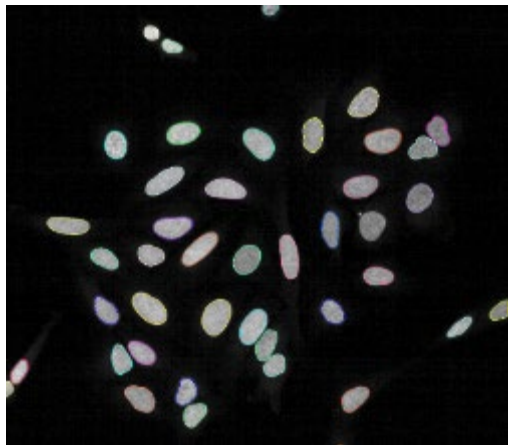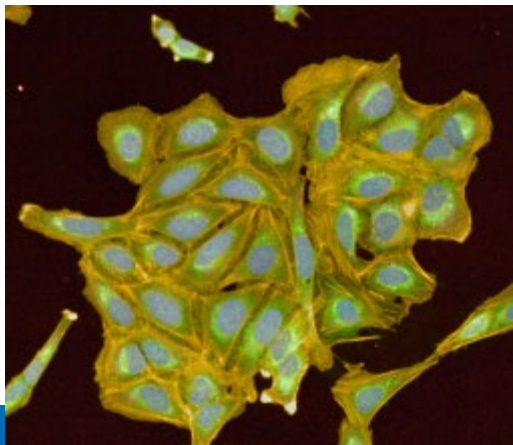
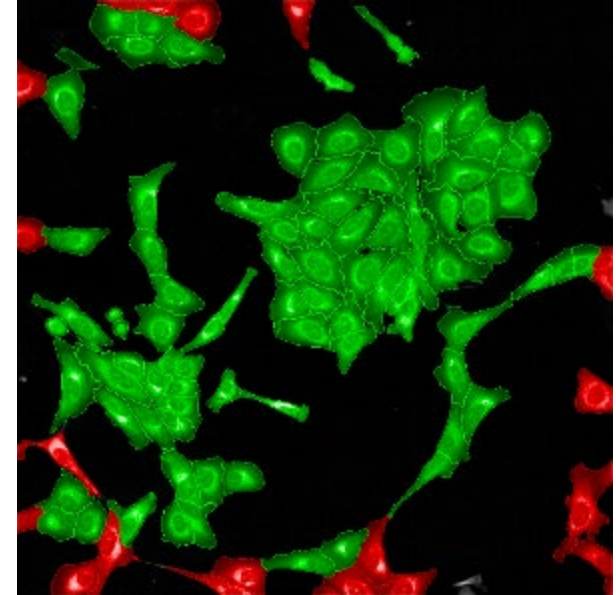# Image Analysis Workflow → Image Segmentation
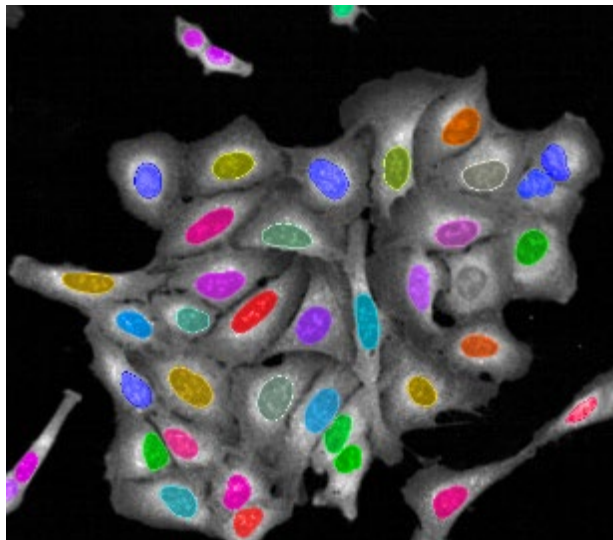
1. find nuclei
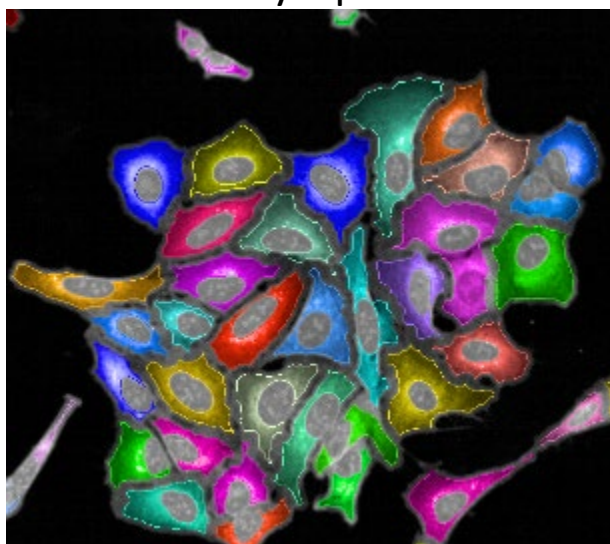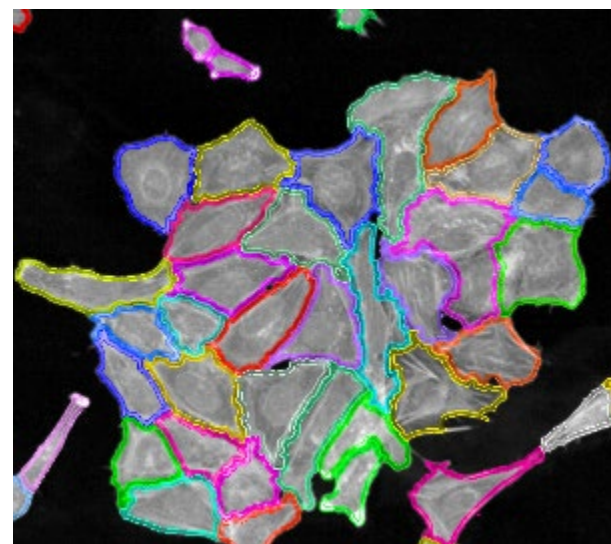
2. find cell outline

3. reject border objects

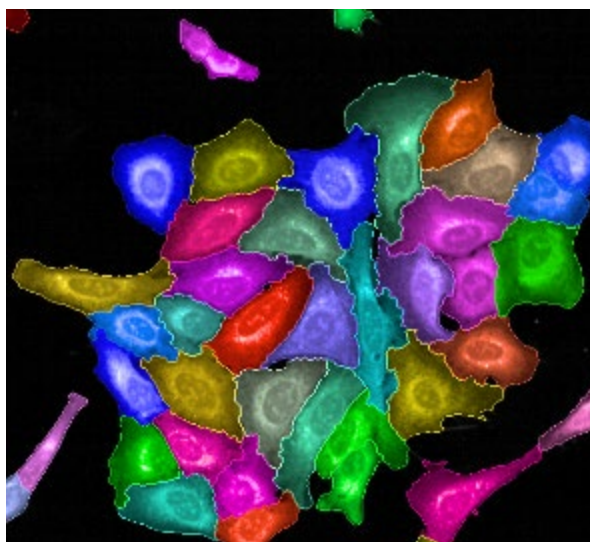# Define Cellular Compartments

nuclei

cytoplasm
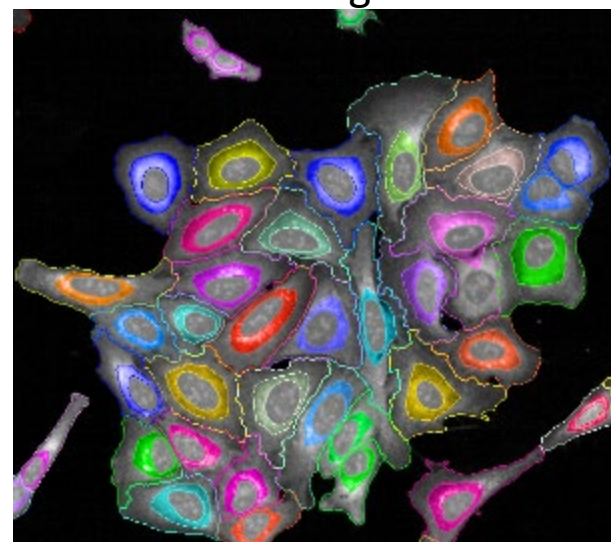
membrane

cell

ring

Phenotypic Feature Extraction
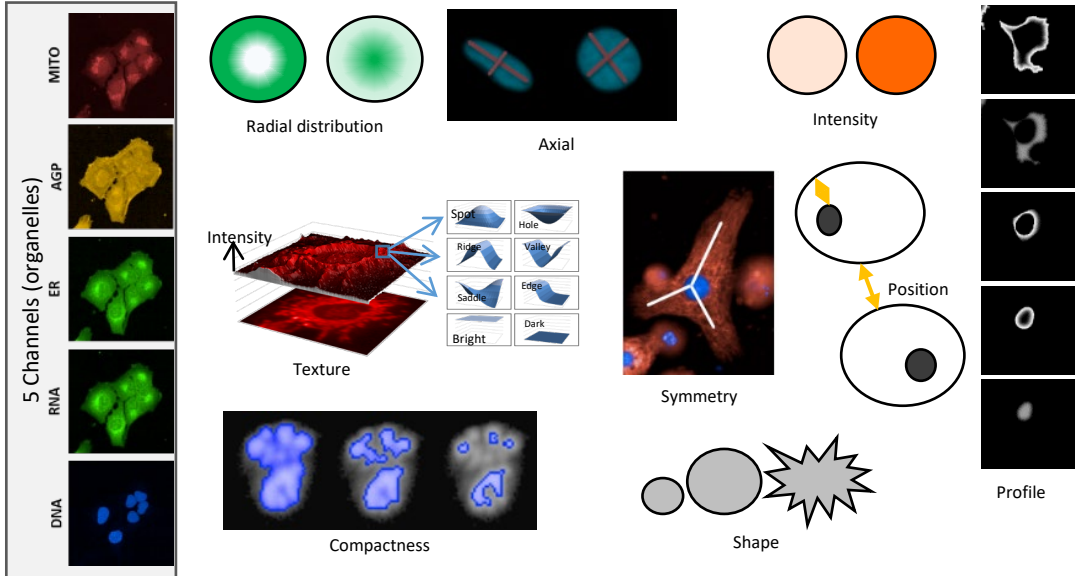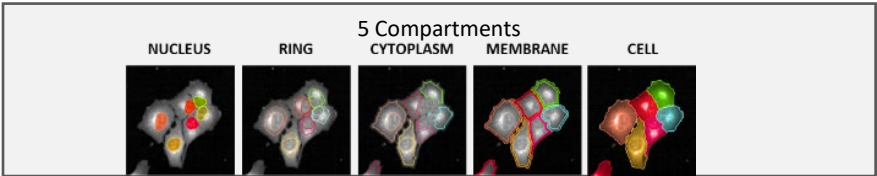
49 Feature Categories
(ex. MITO_Texture_Cytoplasm)

1300 features / cell

PerkinElmer Opera Phenix
Modality: Confocal (single *z*)
Objective: 20X Water
Plate: CellCarrier-384 Ultra
Fields: 5 or 9

| Channel		Module								
		Position [7]	Basic morph-ology [5]	SCARP morphology					Intensity [9]	Texture [14]
				Symmetry [80]	Compactness [40]	Axial [20]	Radial [28]	Profile [20-30]		
	DNA			Nuclei	Nuclei	Nuclei	Nuclei Cell	Nuclei Cytoplasm	Nuclei	Nuclei
	RNA			Nuclei	Nuclei	Nuclei	Nuclei	Nuclei	Nuclei	Nuclei
	ER			Cell	Cell	Cell	Cell	Cytoplasm	Ring Cytoplasm	Ring Cytoplasm
	AGP			Cell	Cell	Cell	Cell	Nuclei Cytoplasm	Ring Cytoplasm Membrane	Ring Cytoplasm Membrane
	Mito			Cell	Cell	Cell	Cell	Nuclei Cytoplasm	Ring Cytoplasm	Ring Cytoplasm
	Not associated with a channel	Nuclei Cell	Nuclei Cell							

Reference Chemical Phenotypes
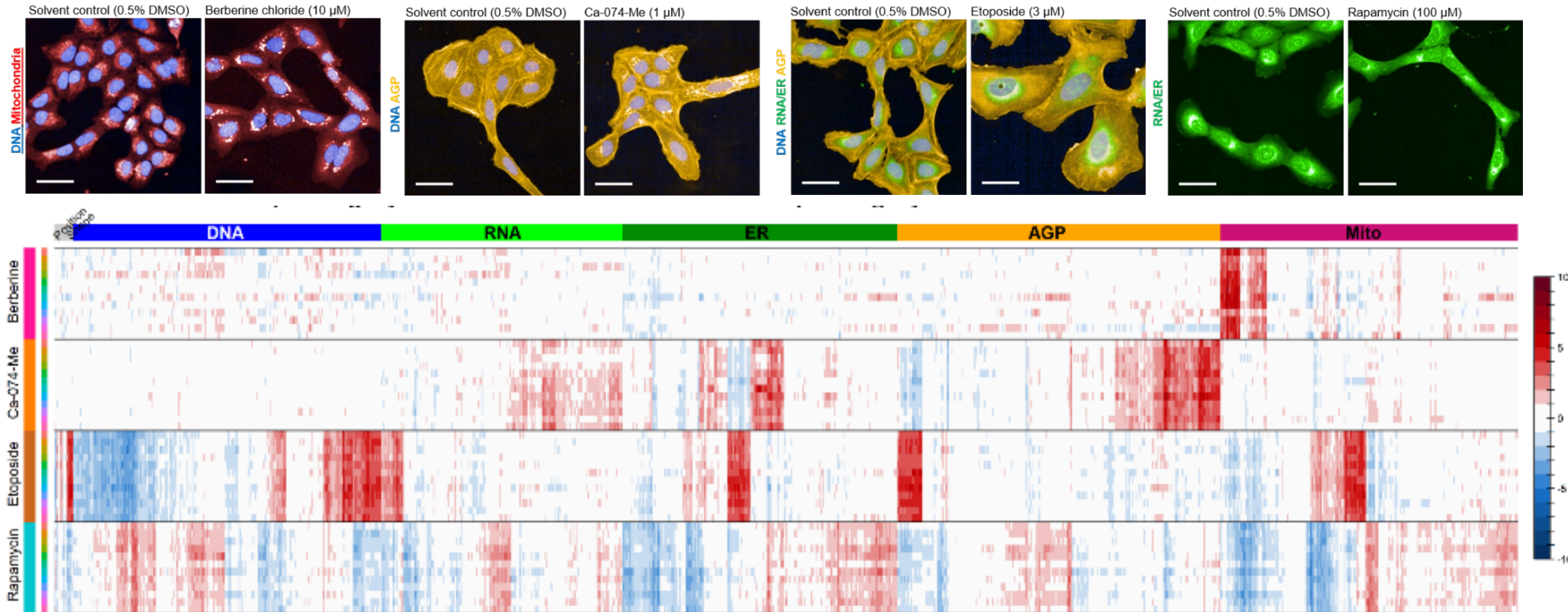
Reference chemicals produce distinct, but reproducible phenotypes in U-2 OS cells.

Concentration Response Modeling of HTPP Data

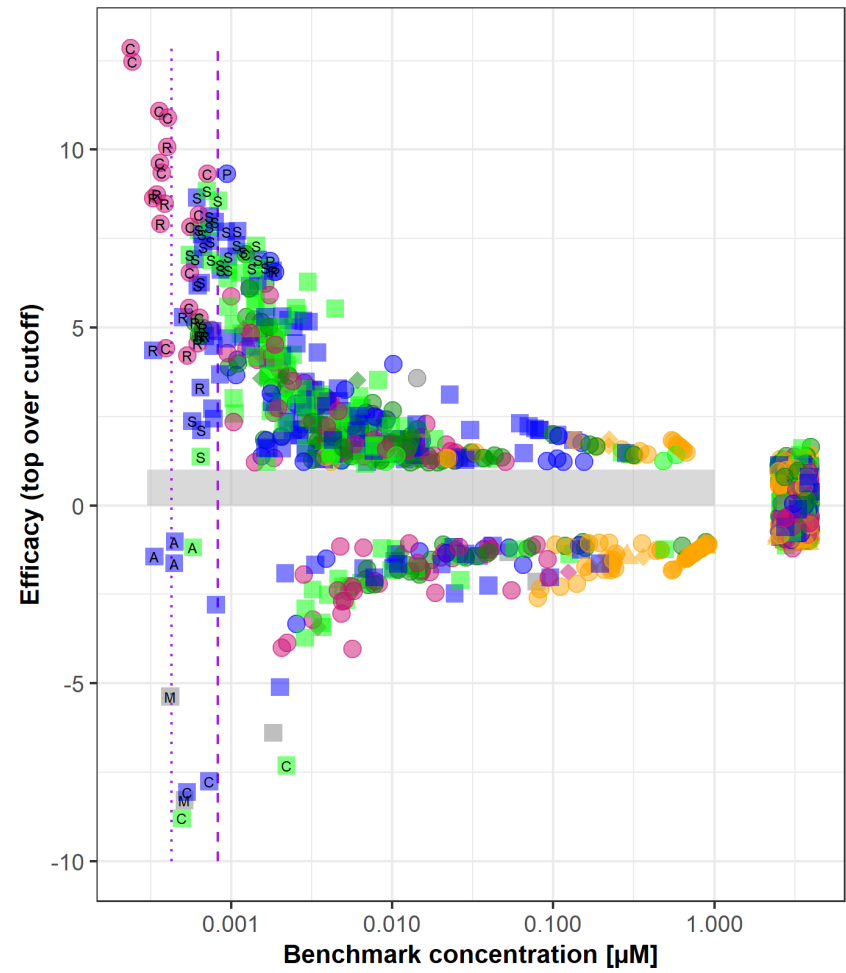**Concentration-response modeling of HTPP data can be performed in a variety of ways.**
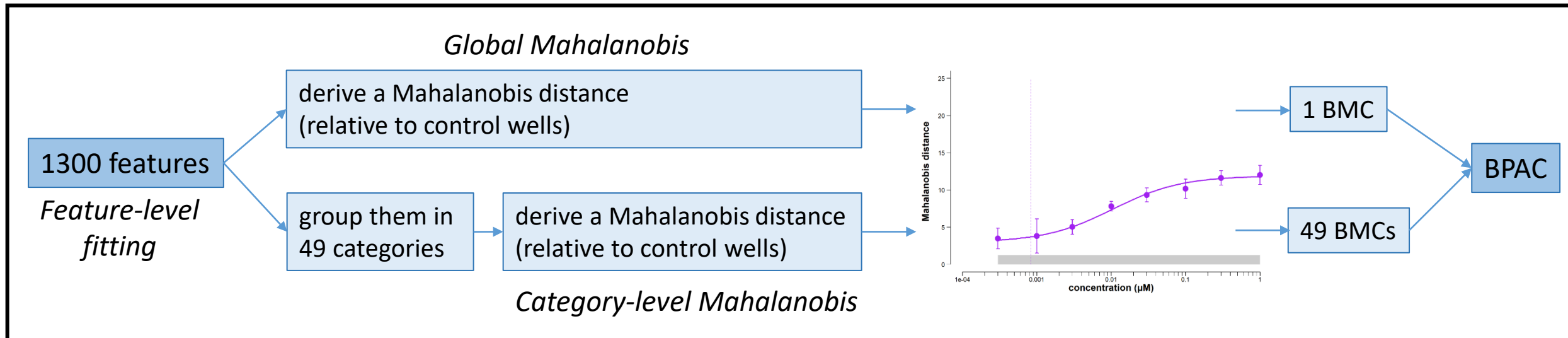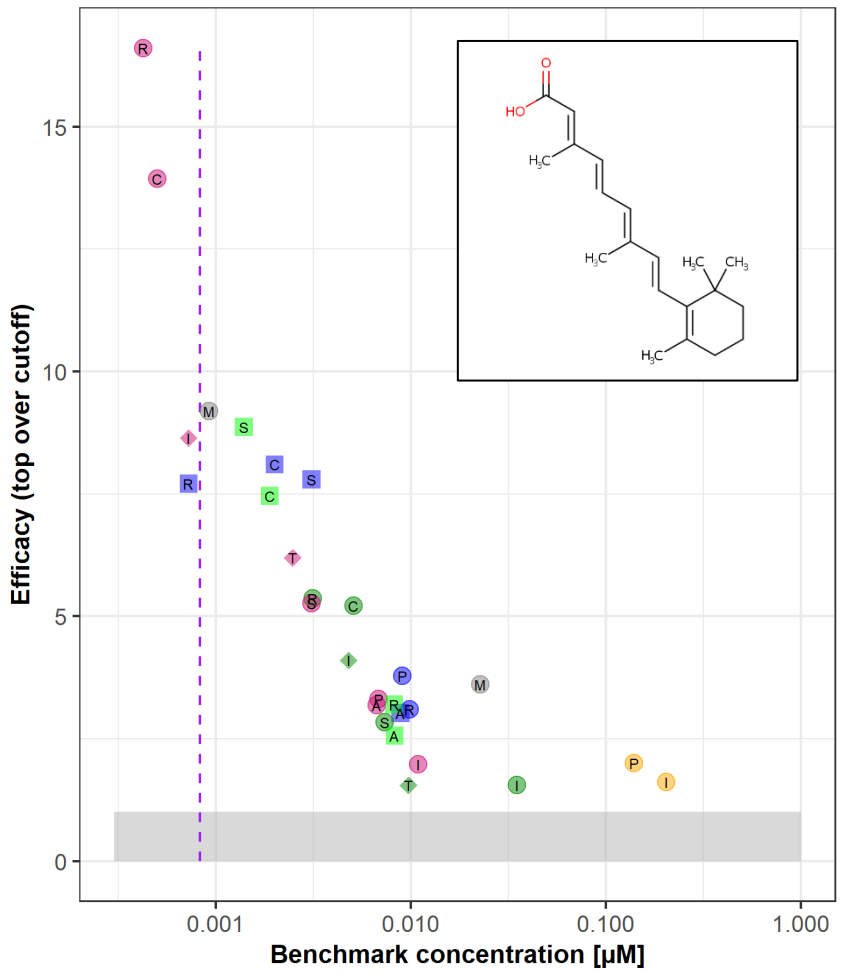

- **Mahalanobis Distance (D_M):** A multivariate distance metric that measures the distance between a point (vector) and a distribution.

- Chemicals where a BMC can be determined using either the global or category D_M approach are considered active.

- The minimum of the global or category BMCs is the **Biological Phenotype Altering Concentration (BPAC).**

Concentration Response Modeling Example Chemical

all-trans-Retinoic acid

DTXSID7021239 | 302-79-4 | RA

2020-07-27
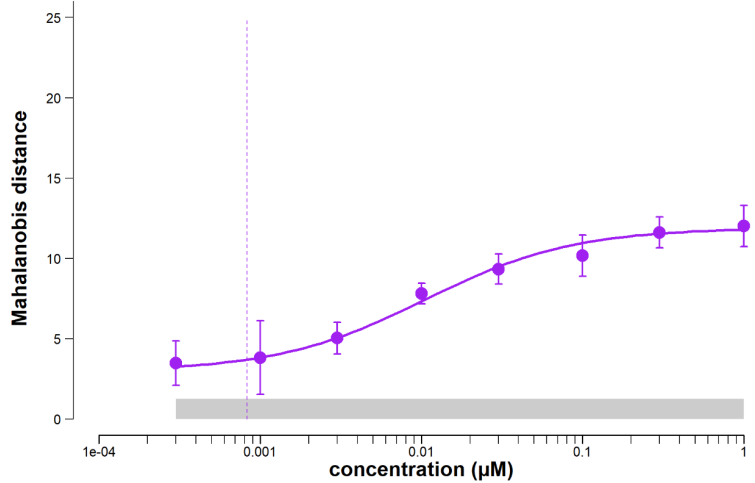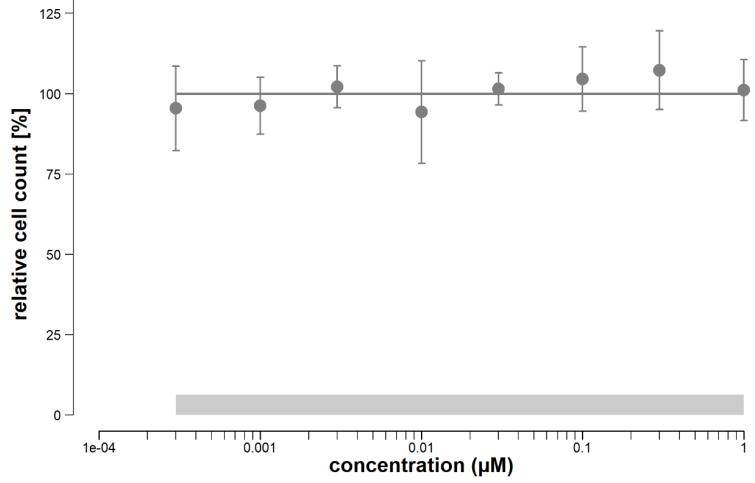
Bioactivity Exposure Ratio (BER) Analysis

Phys-chem Properties $\xrightarrow{\text{predict}}$ Fraction Unbound in Plasma (F_{up})
Intrinsic Clearance (Cl_{int})
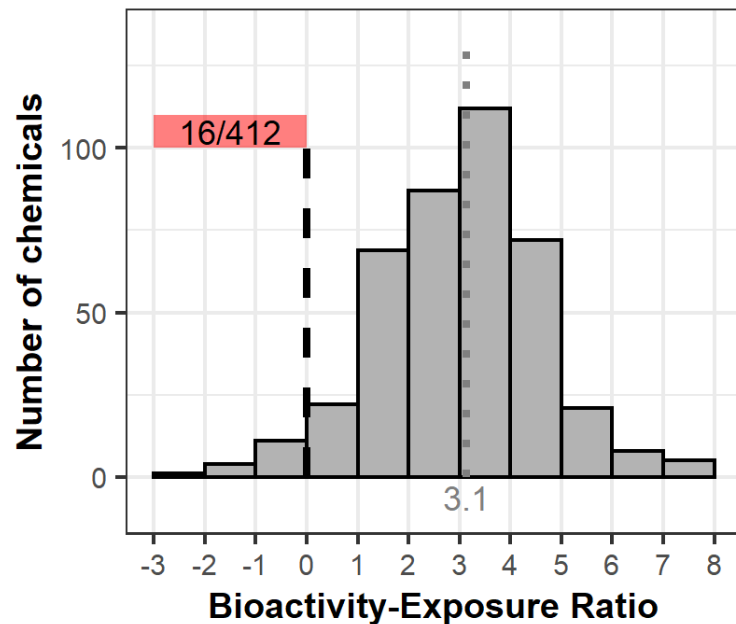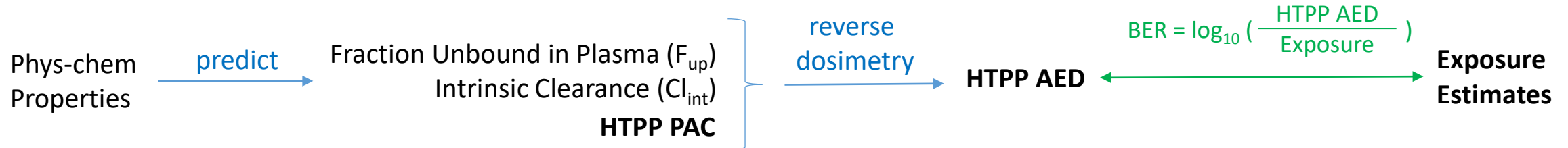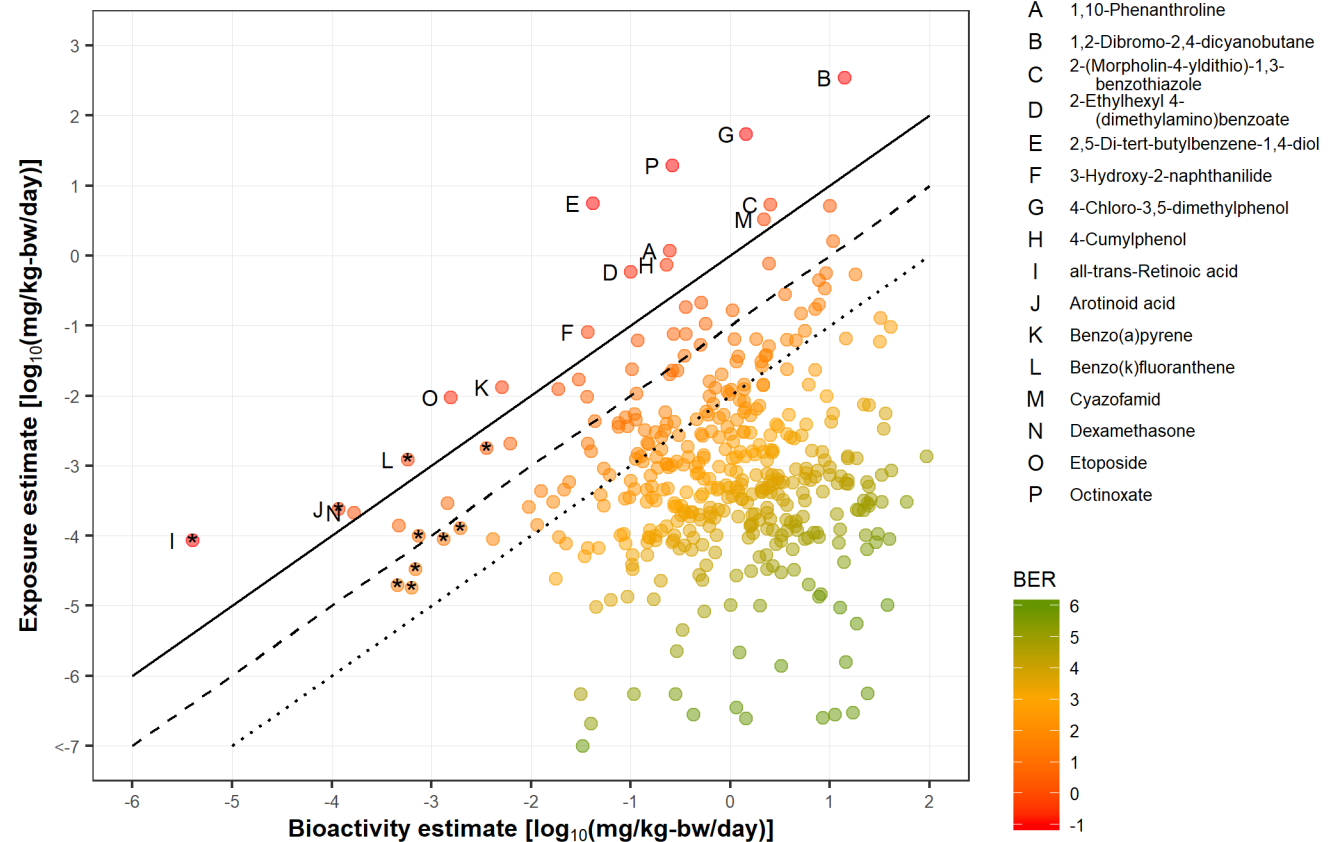**HTPP PAC**

reverse dosimetry $\rightarrow$ **HTPP AED**

$$BER = \log_{10} \left(\frac{HTPP\ AED}{Exposure} \right)$$

HTPP AED $\longleftrightarrow$ **Exposure Estimates**

A	1,10-Phenanthroline
B	1,2-Dibromo-2,4-dicyanobutane
C	2-(Morpholin-4-yldithio)-1,3-benzothiazole
D	2-Ethylhexyl 4-(dimethylamino)benzoate
E	2,5-Di-tert-butylbenzene-1,4-diol
F	3-Hydroxy-2-naphthanilide
G	4-Chloro-3,5-dimethylphenol
H	4-Cumylphenol
I	all-trans-Retinoic acid
J	Arotinoid acid
K	Benzo(a)pyrene
L	Benzo(k)fluoranthene
M	Cyazofamid
N	Dexamethasone
O	Etoposide
P	Octinoxate

16 Chemicals were identified were biological activity overlaps with predicted human exposure.

Figures courtesy of J Nyffeler

Phenotypic Profile Similarity with Nuclear Receptor Modulators

Biological similarity in HTPP

Gene expression in U-2 OS

target

AR
BAR
CAR
ESR
GR
PGR
PPAR
PXR
RAR
VDR
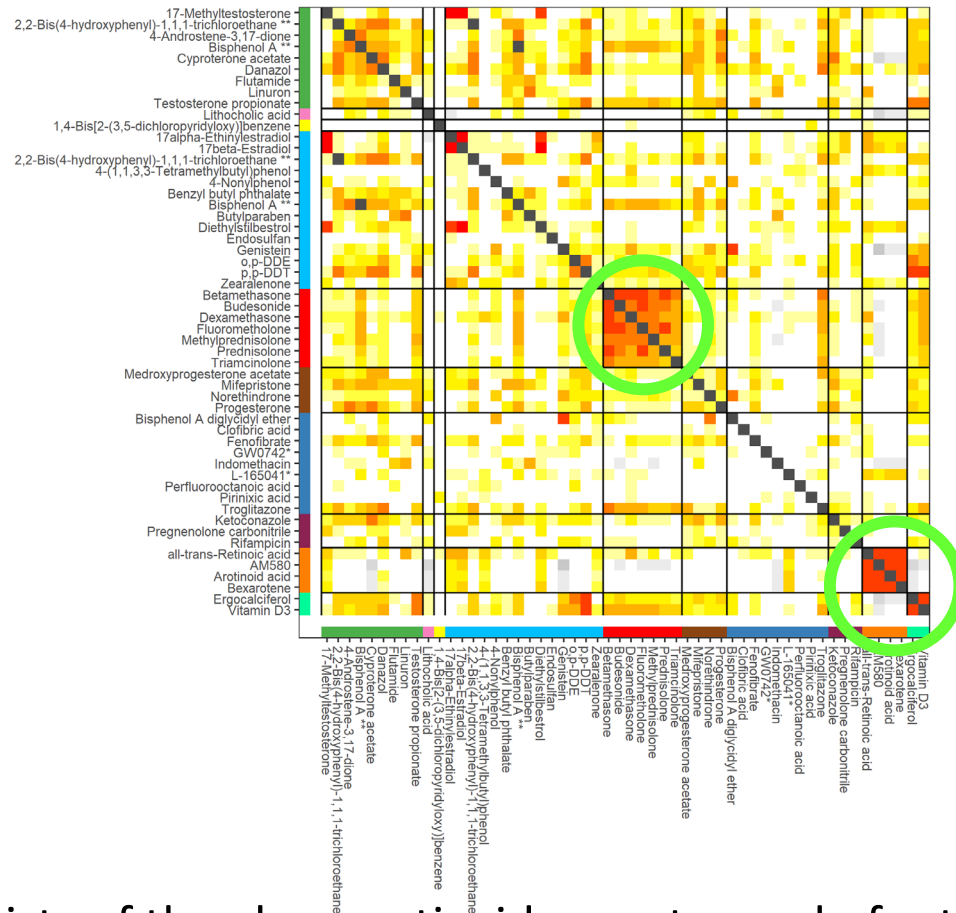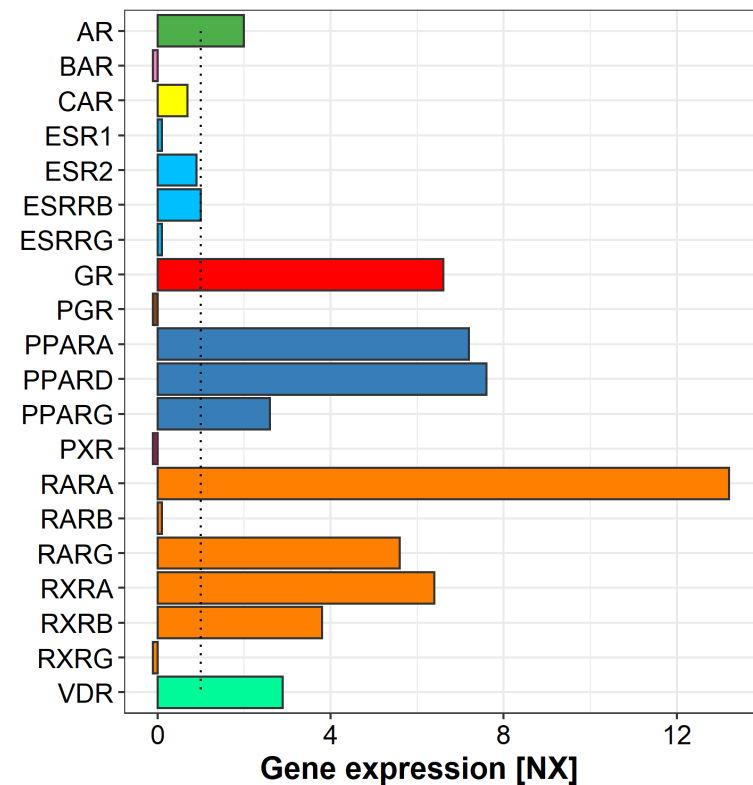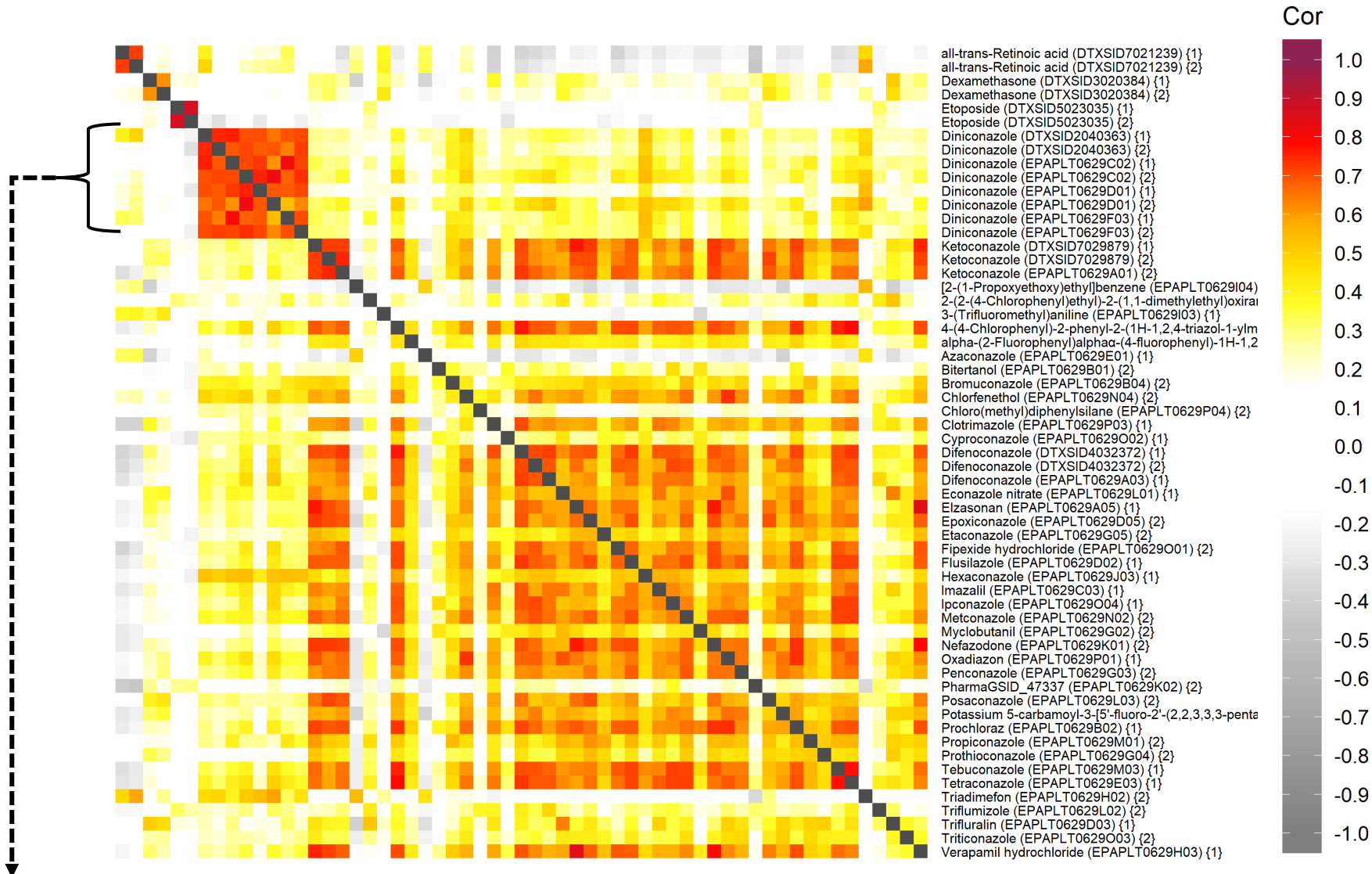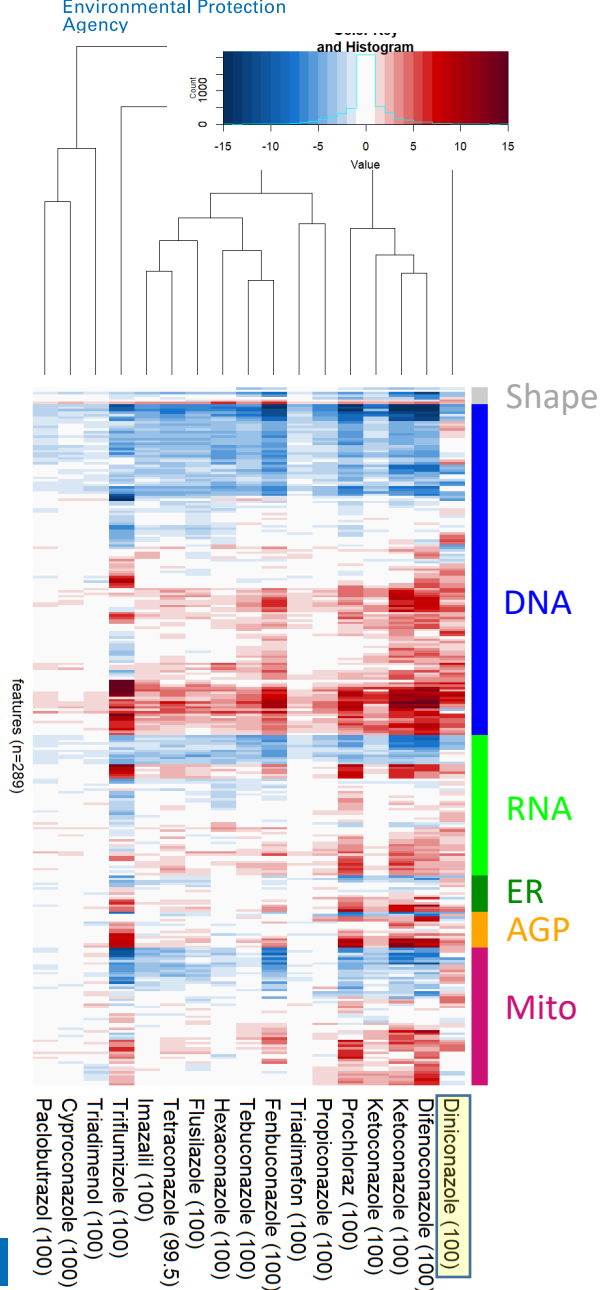
Agonists of the glucocorticoid receptor and of retinoic acid receptors display characteristic profiles

Expression of a target does not guarantee that characteristic profiles are observed (e.g. PPAR)

Figures courtesy of J Nyffeler

Phenotypic Profile Similarity of Conazoles

The Diniconazole phenotype is distinct from most other conazoles.
Informs biological activity-based chemical read across.

Figures courtesy of J Nyffeler

Summary and Conclusions

- **HTPP Screening:** We have established robust and scalable laboratory and bioinformatics workflow for transcriptomics and phenotypic screening of environmental chemicals in human-derived cell lines.

- **Assay Reproducibility:** We have demonstrated a high degree of assay reproducibility for HTPP screening assays through the use of reference chemicals.

- **Bioactivity to Exposure Ratio:** Biological phenotype altering concentrations (BPACs) can be converted to administered equivalent doses (AEDs) and compared to human exposure predictions for chemical ranking and prioritization.

- **Mechanistic Prediction:** Phenotypic profiles can be used to discern mechanisms of action by comparison to reference chemicals or comparison to chemicals within the same molecular class.

- **Future Work:** Expand the amount of biological space evaluated for environmental chemicals by screening in additional, complementary cell types.

Acknowledgements

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