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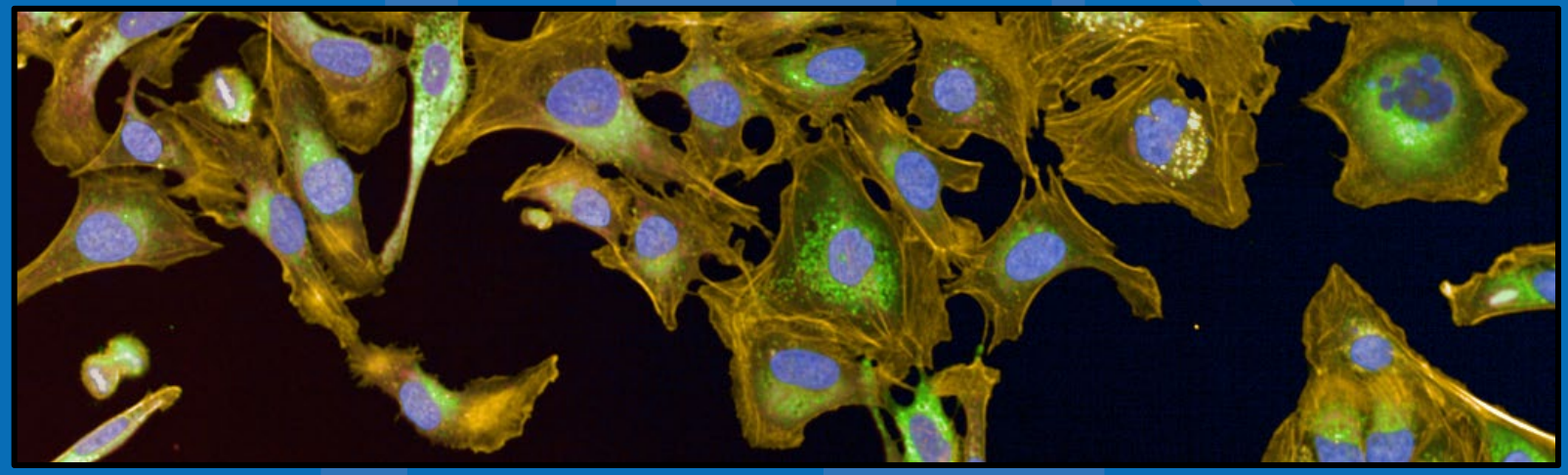
Application of Cell Painting, an Imaging-Based High Throughput Phenotypic Profiling Assay for Bioactivity Screening of Environmental Chemicals

Johanna Nyffeler

ORISE postdoctoral grantee at USEPA Center for Computational Toxicology and Exposure (CCTE)



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Office of Research and Development
Center for Computational Toxicology & Exposure

Society of Toxicology (SOT) Annual Meeting
March 22nd, 2021

Conflict of Interest Statement

The author declares no conflict of interest.

The views expressed in this presentation are those of the author and do not necessarily reflect the views or policies of the U.S. EPA.

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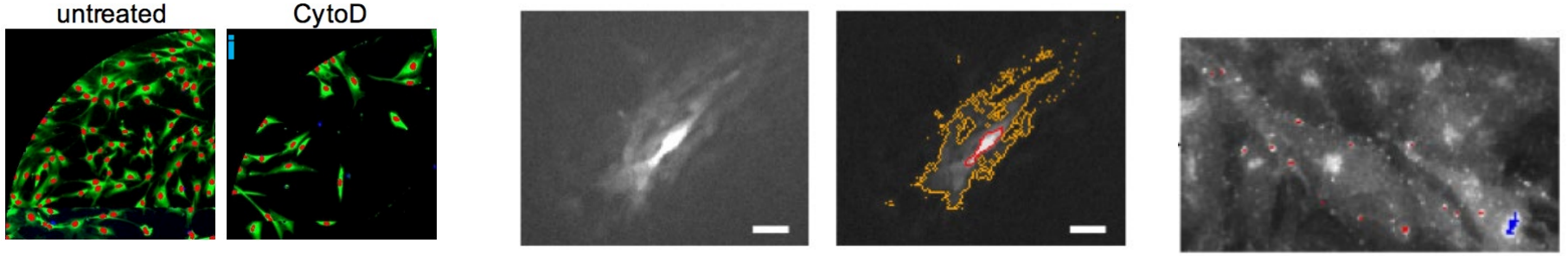
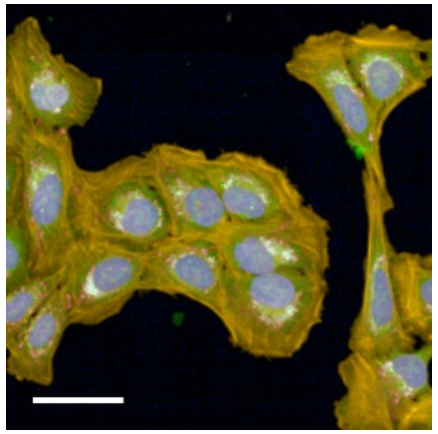
Introduction: Dr. Johanna Nyffeler

- BSc in Biochemistry, MSc in Genetics
- **PhD at University of Konstanz, Germany**
  - group of Dr. Marcel Leist
  - development of high-content assays for *in vitro* developmental neurotoxicology



- **PostDoc at Center for Computational Toxicology & Exposure (CCTE), US EPA**
  - group of Dr. Joshua Harrill
  - high-throughput image-based profiling ('Cell Painting'), computational toxicology



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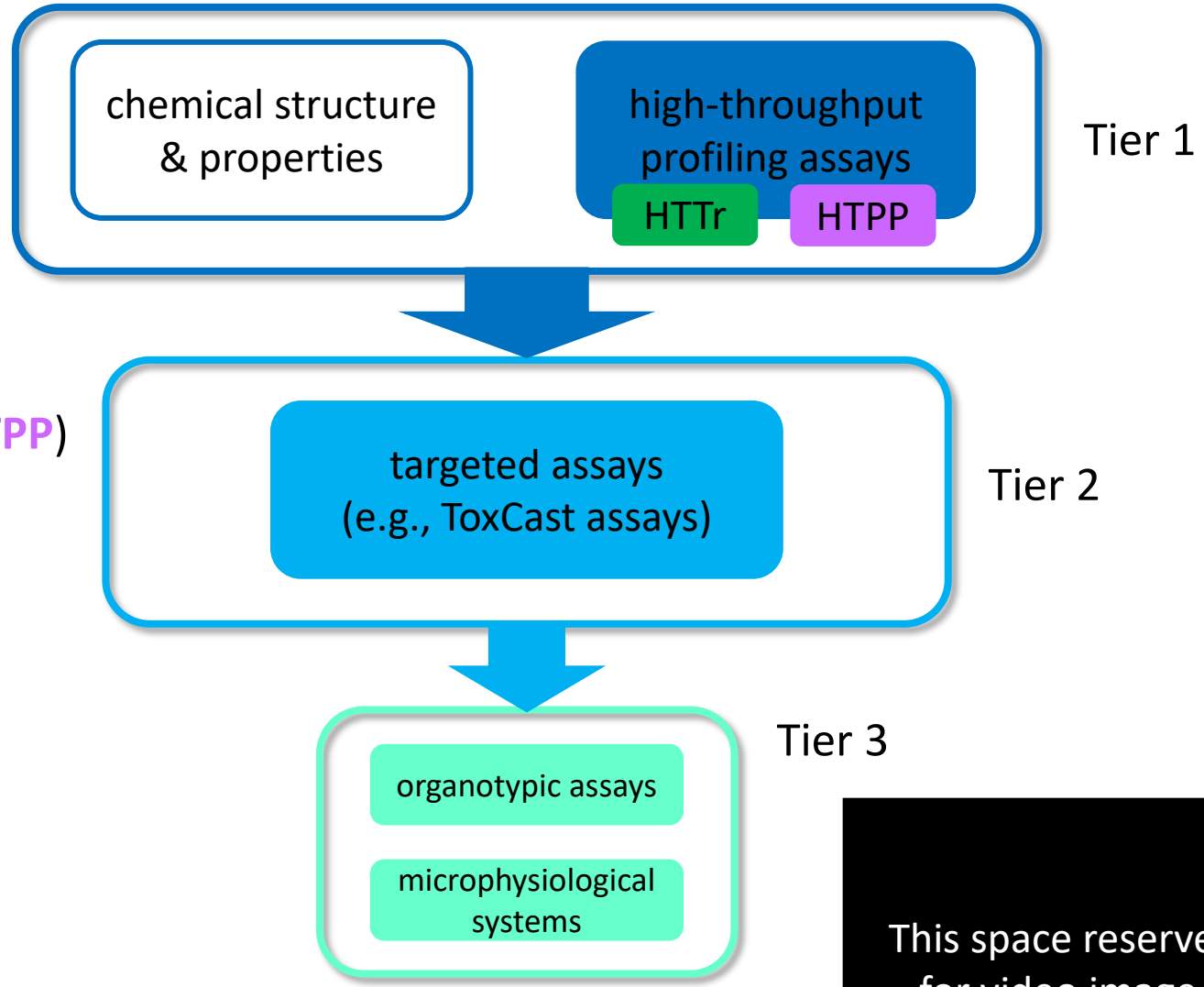
Tiered Hazard Evaluation Strategy based on New Approach Methods (NAMs)

Profiling Assays

- untargeted
- measure large number of endpoints (e.g., transcripts, phenotypic features)
- high-throughput transcriptomics (**HTTr**) (Harrill et al. 2021, PMID: 33538836)
- high-throughput phenotypic profiling (**HTPP**) (Nyffeler et al. 2020, PMID: 31899216)

Goals

- potency estimation
- mechanistic prediction



adapted from "The Next Generation Blueprint of Computational Toxicology at the U.S. EPA", Tox. Sci. 2019; 169(2):317-322. PMID: 30835285

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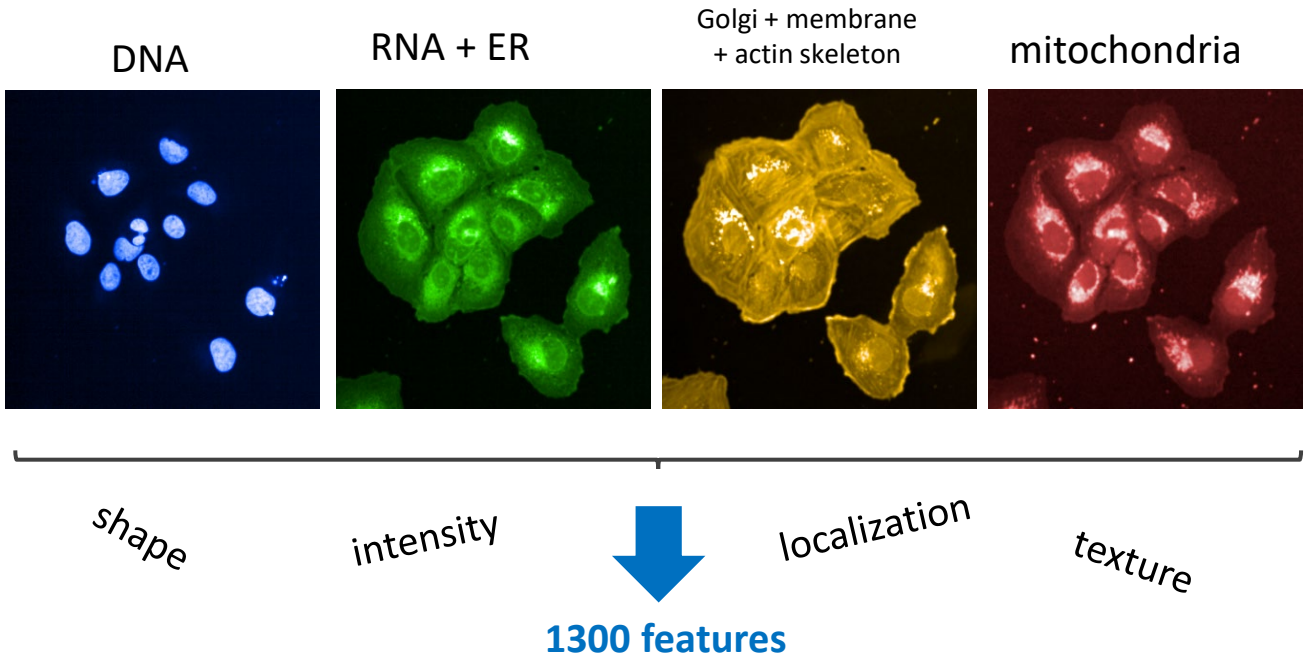
What is Imaging-Based Phenotypic Profiling?

- labeling of various cell organelles with fluorescent probes in *in vitro* cultures
- assessing a large variety of morphological features on individual cells

'Cell Painting' assay

Gustafsdottir *et al.* 2013

Bray *et al.* 2016



Flourescent labels
DNA: H-33342
RNA: SYTO14
ER: Concanavalin A-488
Actin: Phalloidin-568
Golgi + Membrane: wheat germ agglutinin (WGA) -555
Mitochondria: MitoTracker

Nyffeler *et al.* 2020

Cell Painting = Phenotypic Profiling

High-Throughput Phenotypic Profiling = HTPP

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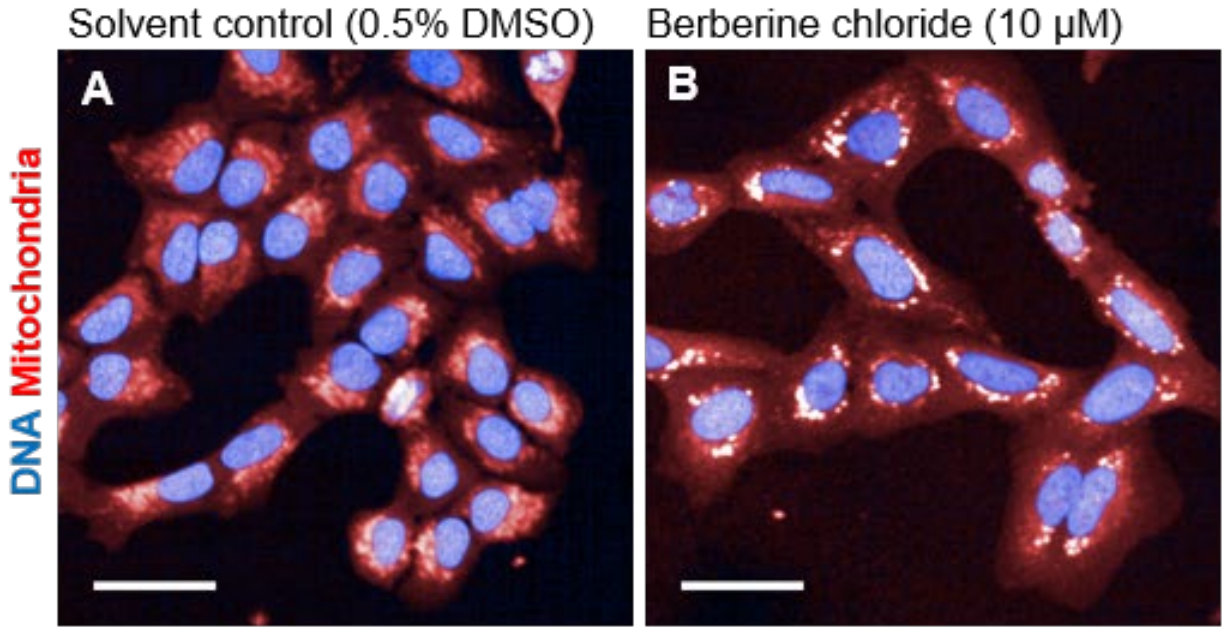
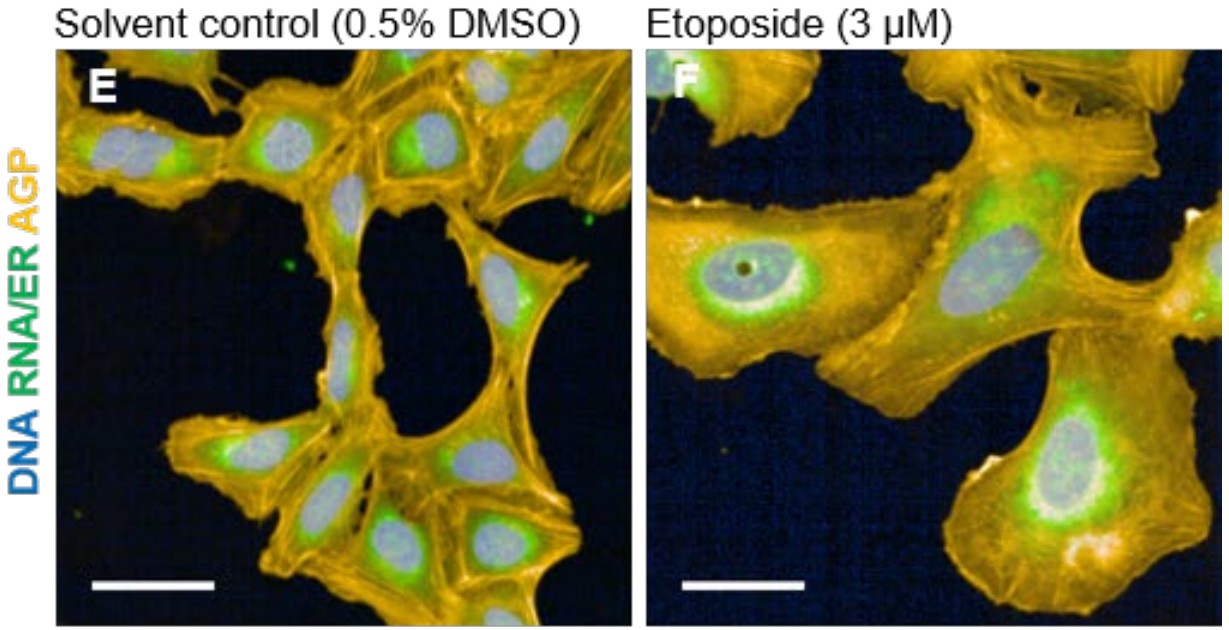
Example Chemicals: Qualitative Observation



→ Mitochondrial compactness/texture



→ Cells are larger

⇨ **Strong phenotypes are observable qualitatively**

adapted from Nyffeler *et al.* 2020

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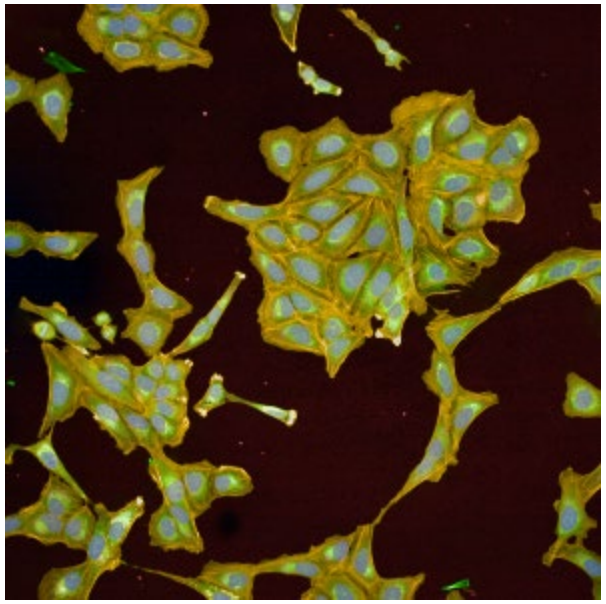
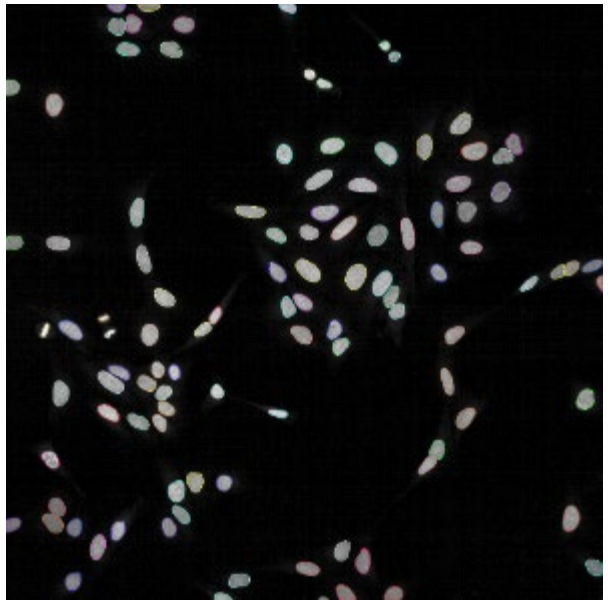
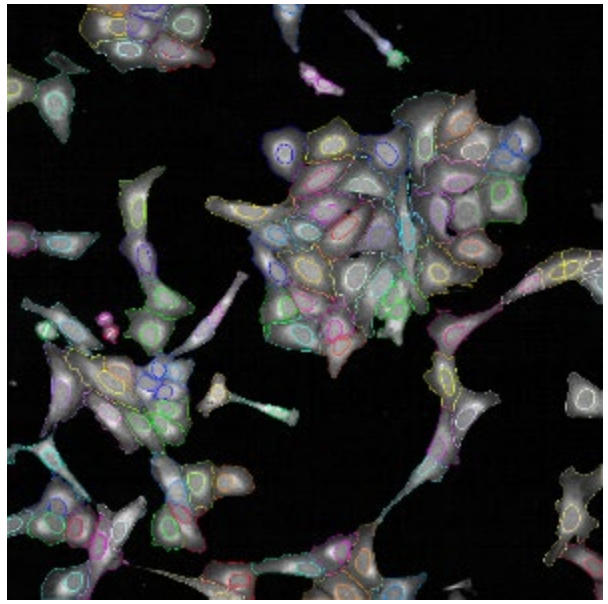
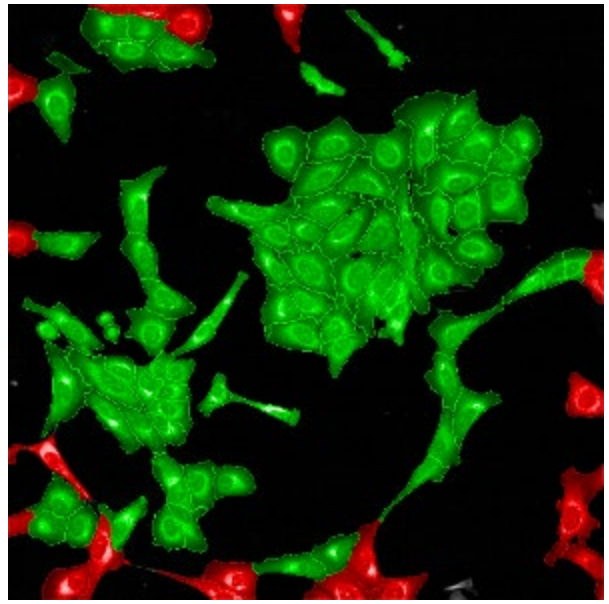
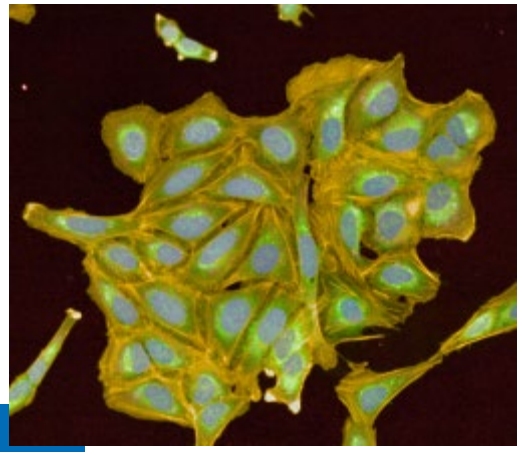
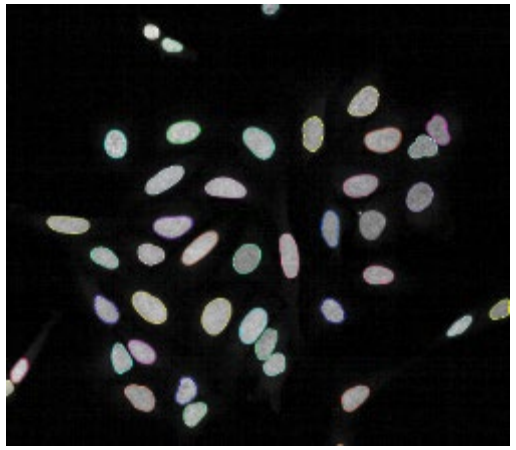
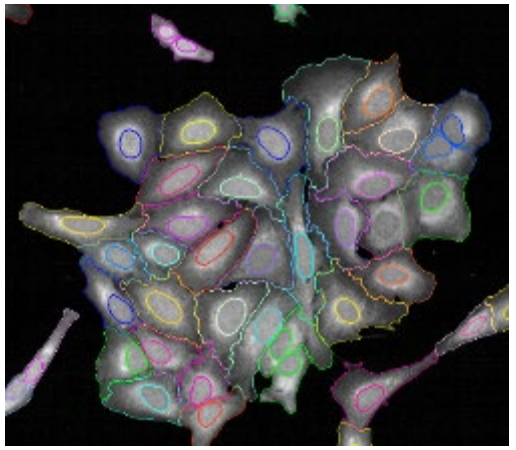
Image Analysis Workflow → Image Segmentation

1. find nuclei

2. find cell outline

3. reject border objects

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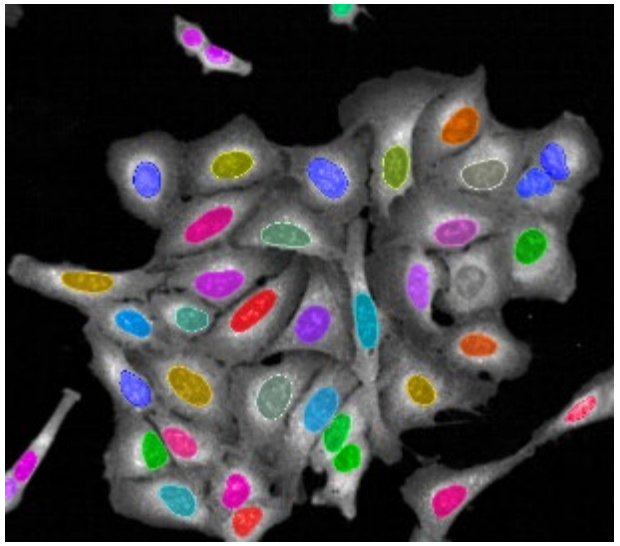
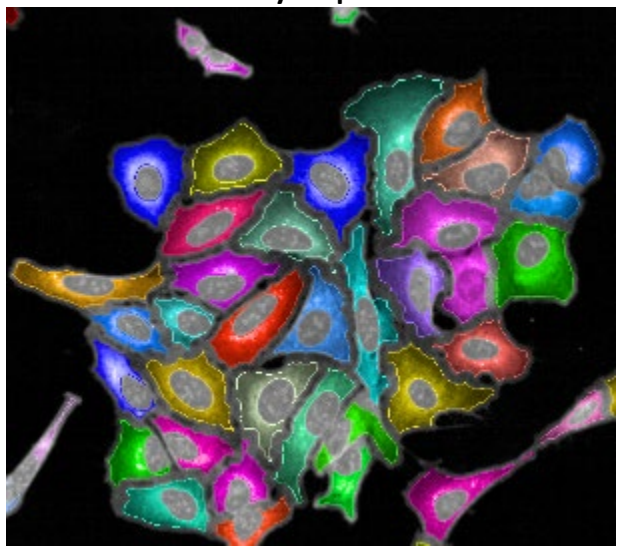
Define Cellular Compartments

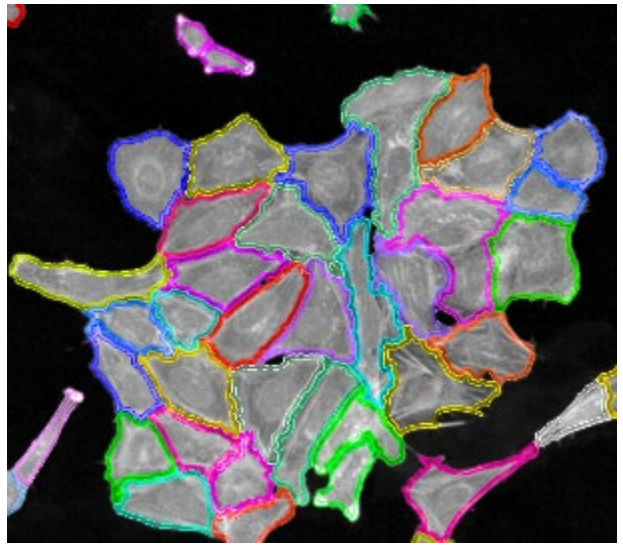
nuclei



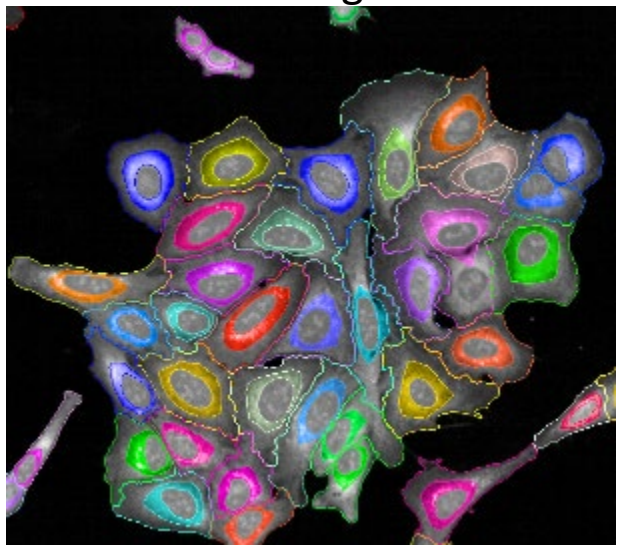
cytoplasm



membrane



ring



cell

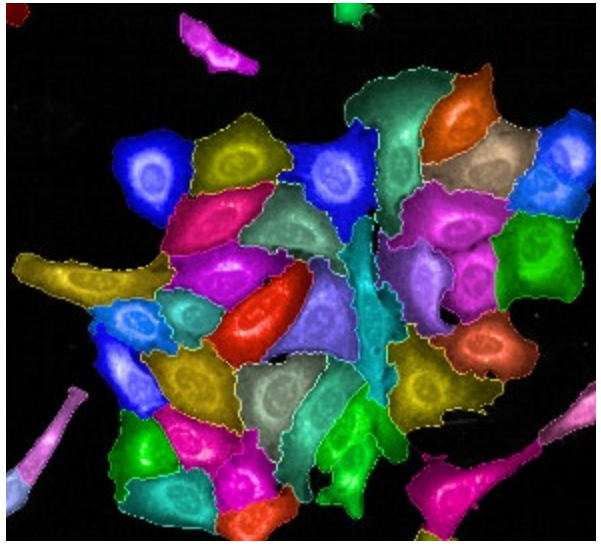


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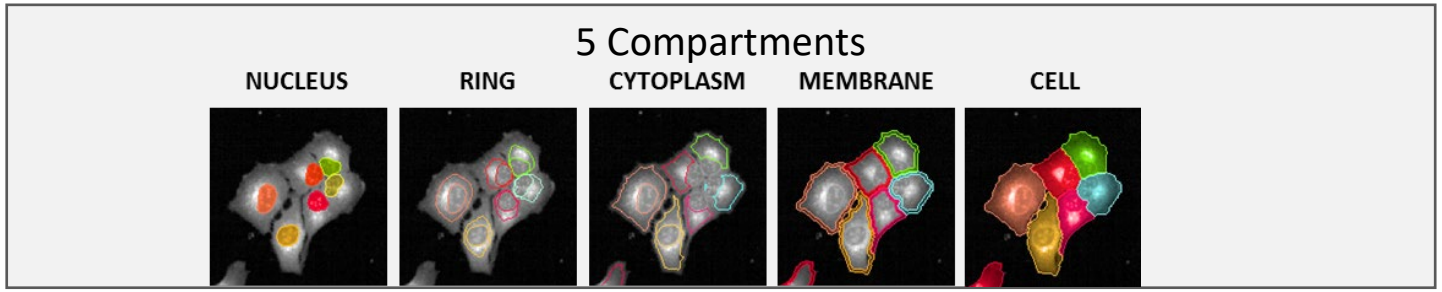
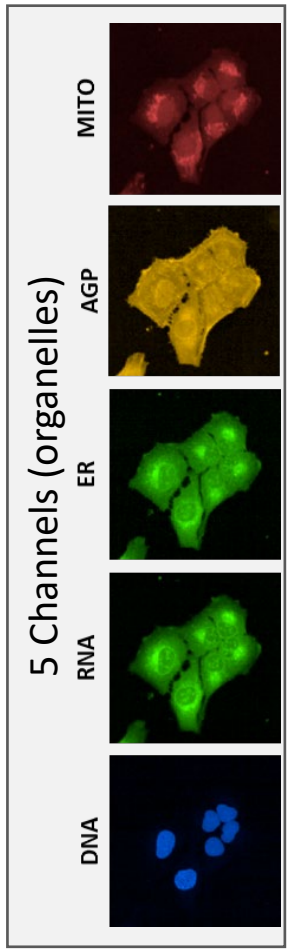
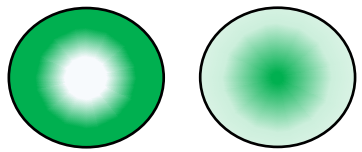
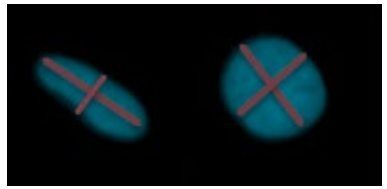
Image Processing

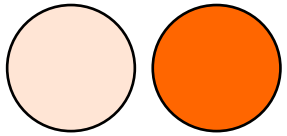
Profiling
with Perkin Elmer
Harmony Software

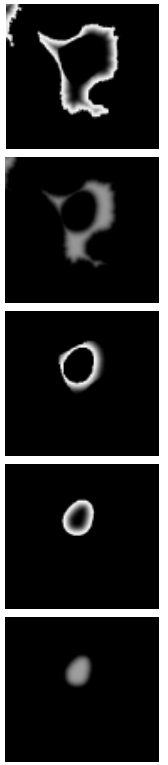


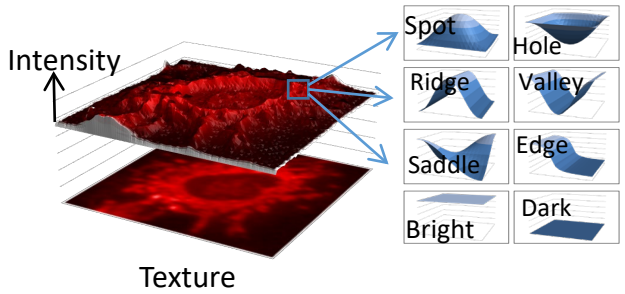



Radial distribution

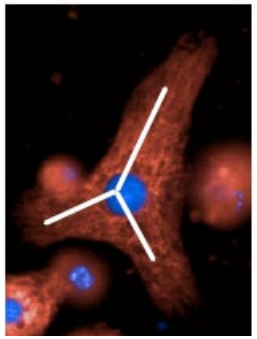

Axial

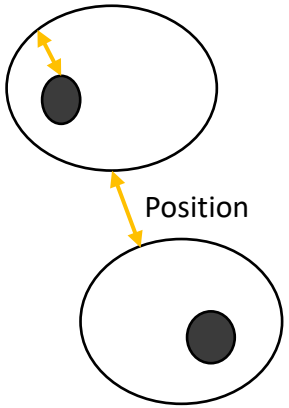

Intensity

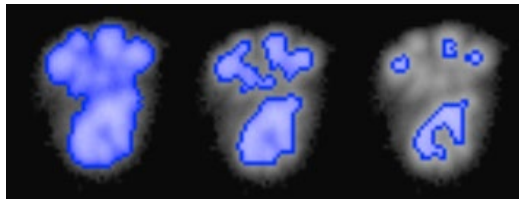

Profile

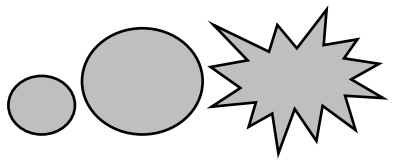


Texture


Symmetry




Compactness


Shape

= 1300 features

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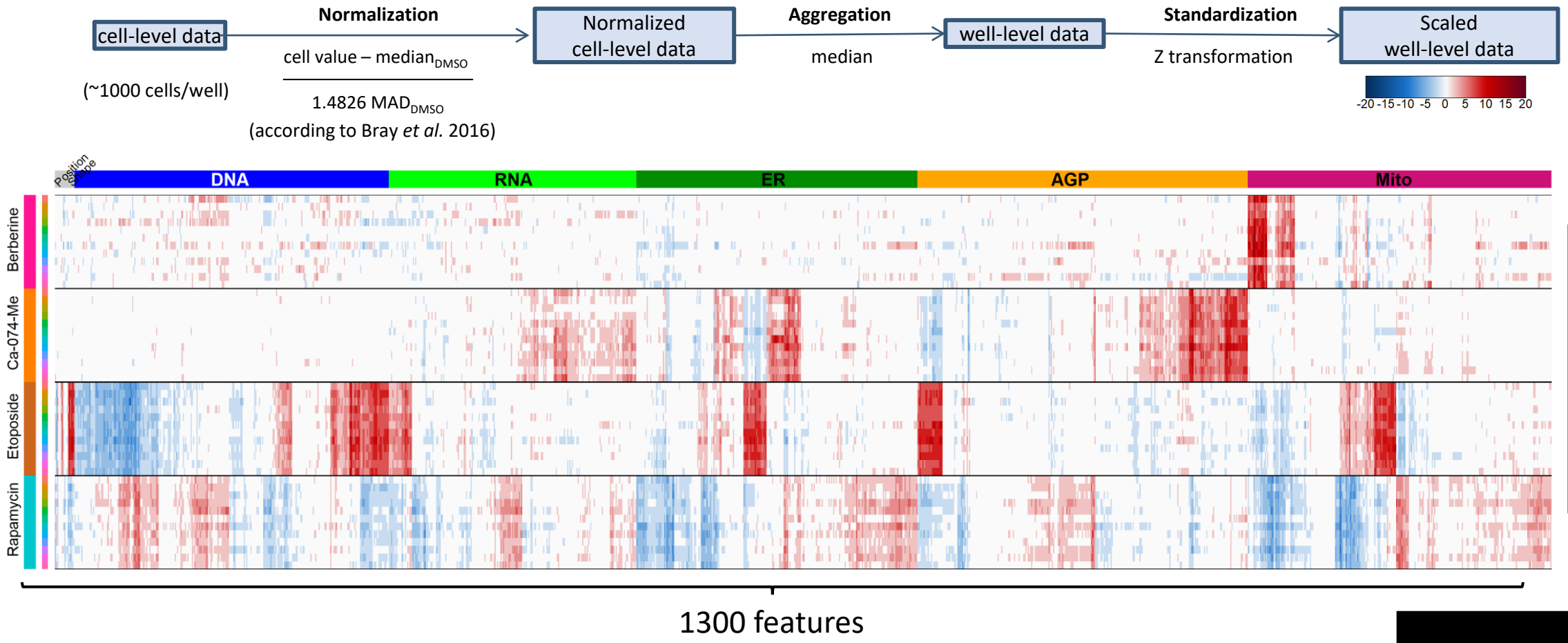
With illustrations from Perkin Elmer

Example Chemicals: Quantitative Observation



⇨ **Qualitative observations can be quantified**

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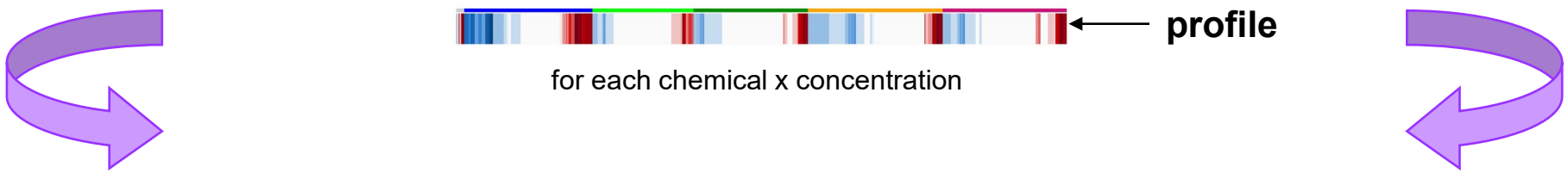
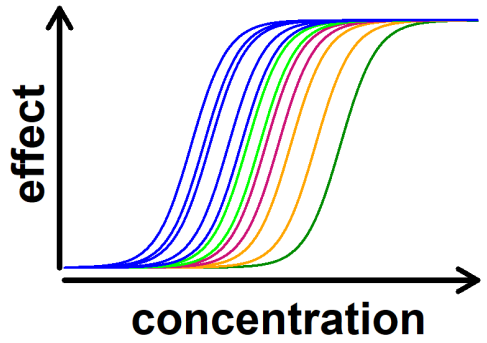
adapted from Nyffeler *et al.* 2020

Two Applications



Application 1

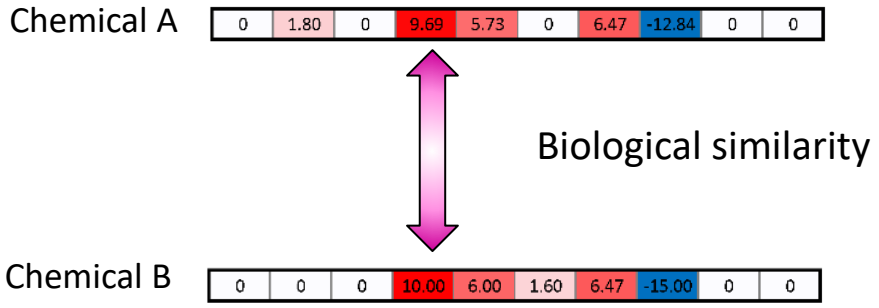
concentration-response modelling



Potency estimation:
***in vitro* point-of-departure (POD)**

- Nyffeler *et al.* (2020) Toxicol Appl Pharmacol. PMID: 31899216
- Willis *et al.* (2020). SLAS Discov. PMID: 32546035
- Nyffeler *et al.* (2021). SLAS Discov. PMID: 32862757

Application 2



Compare profiles with annotated reference chemicals
→ putative mechanisms

work in progress

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U-2 OS ToxCast Screen Experimental Design

Parameter	Multiplier	Notes
Cell Type(s)	1	U-2 OS
Time Points:	1	24 hours
Chemicals	1,202	TSCA Chemicals of interest to US EPA • Includes 462 APCRA case study chemicals • Includes 179 chemicals with annotated molecular targets
Concentrations:	8	3.5 log_{10} units; ~half-log_{10} spacing
Biological Replicates:	4	--

APCRA
ACCELERATING THE PACE OF CHEMICAL RISK ASSESSMENT

Kavlock et al. (2018)
Chem. Res. Tox; 31(5): 287-290

International collaboration of regulatory scientists focused on next generation chemical risk assessment including **deriving quantitative estimates of risk based on NAM-derived potency information and computational exposure estimates.**

APCRA Chemicals → PK parameters necessary for *in vitro* to *in vivo* extrapolation (IVIVE) *in vivo* toxicity data

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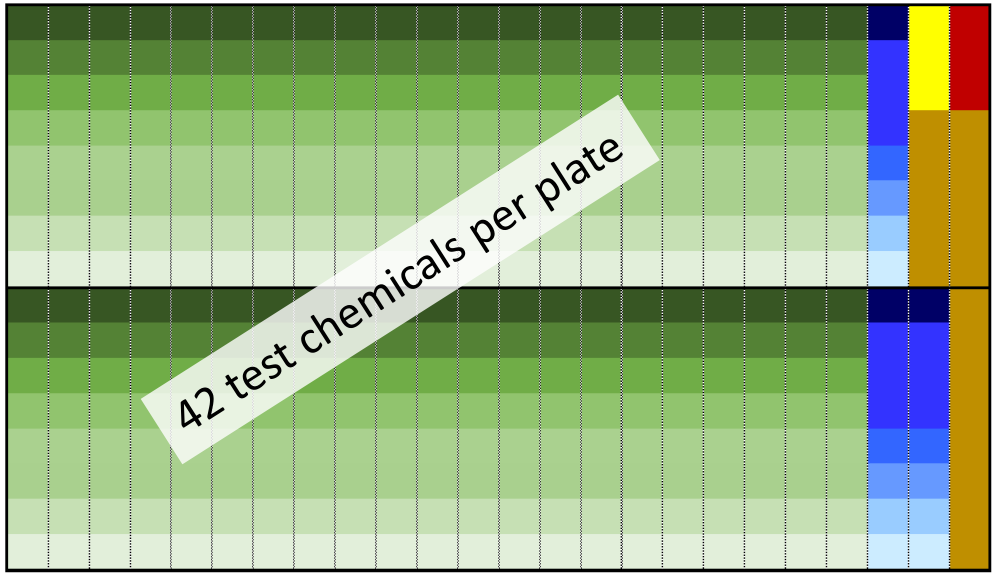
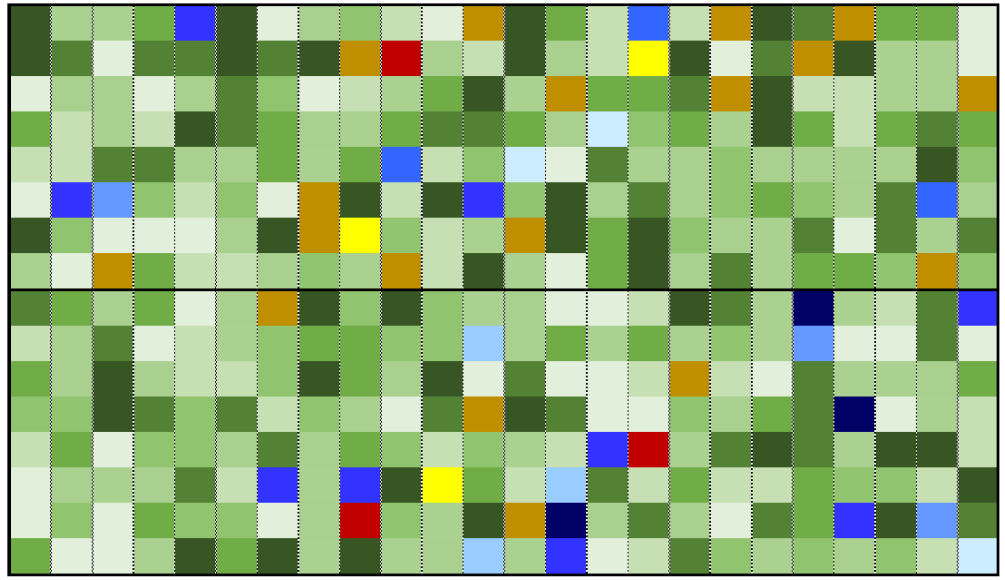
U-2 OS ToxCast Screen Dose Plate Design





Label	Reference Chemicals:	Molecular Mechanism-of-Action	Test Concentrations
A	Etoposide	DNA topoisomerase inhibitor	0.03 - 10 µM
B	all-trans-Retinoic Acid	Retinoic acid receptor agonist	0.0003 – 1 µM
C	Dexamethasone	Glucocorticoid receptor agonist	0.001 – 3 µM
D	Trichostatin A	Histone deacetylase inhibitor	1 µM
E	Staurosporine	Cytotoxicity control	1 µM
F	DMSO	Vehicle control	0.5 %

each test plate is uniquely randomized
→ no systematic edge effects

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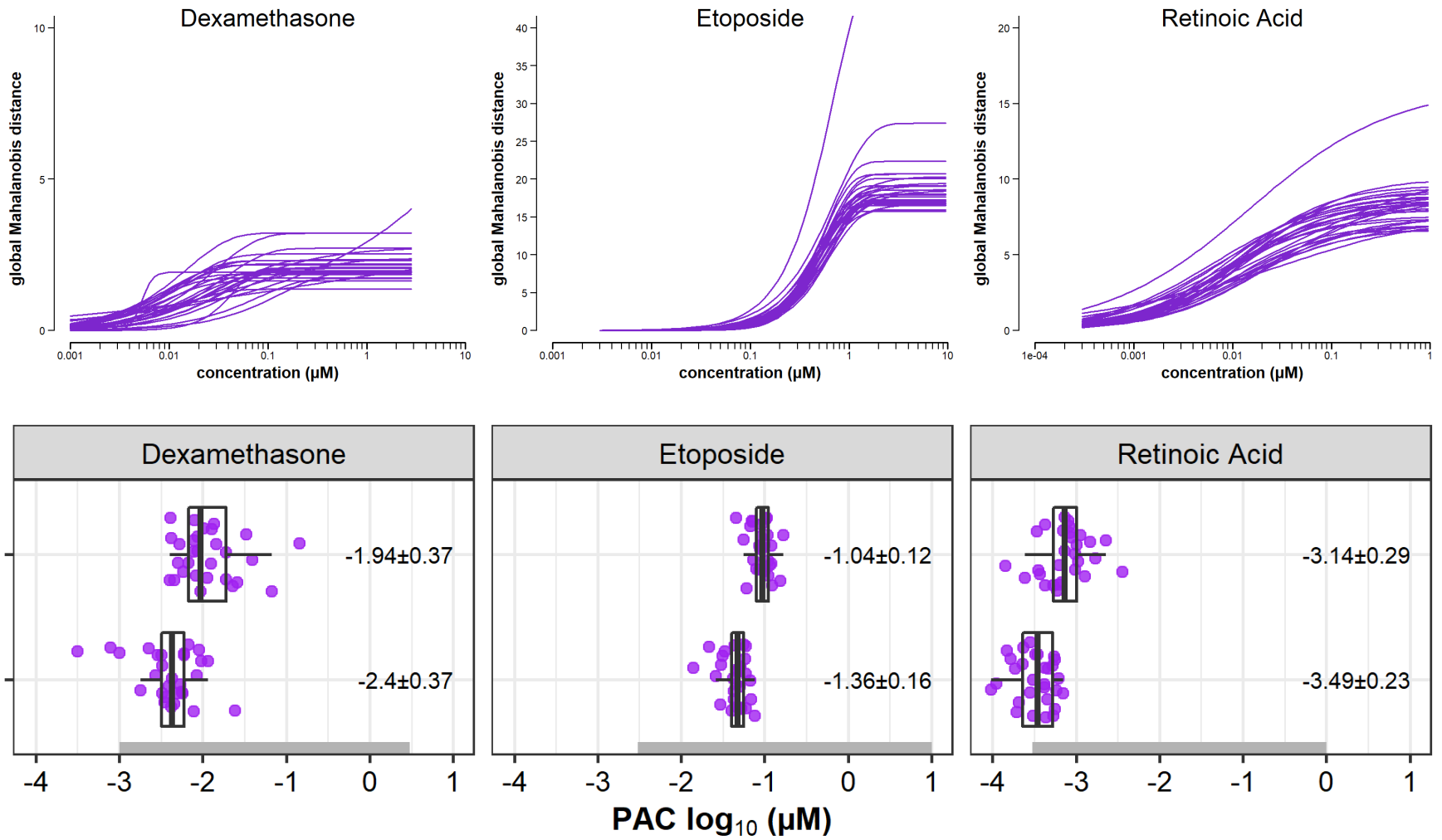
Reproducibility: Potencies



⇨ **Potency estimates vary less than ½ an order of magnitude**

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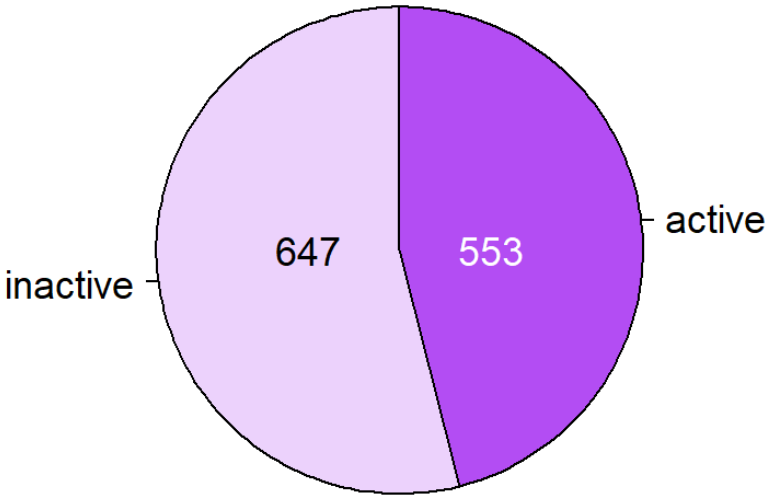
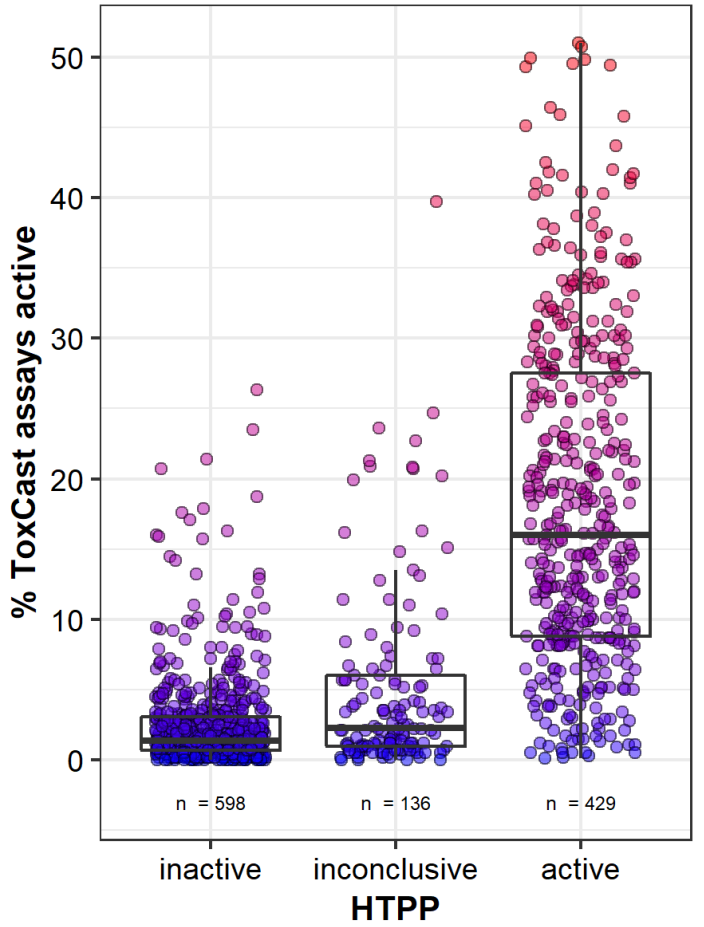
HTPP Screening Results (1)

Active chemicals:





- ~ 40% of chemicals were active
- Most activity is > 10 µM
- Chemicals active in HTPP are more often 'promiscuous' in ToxCast

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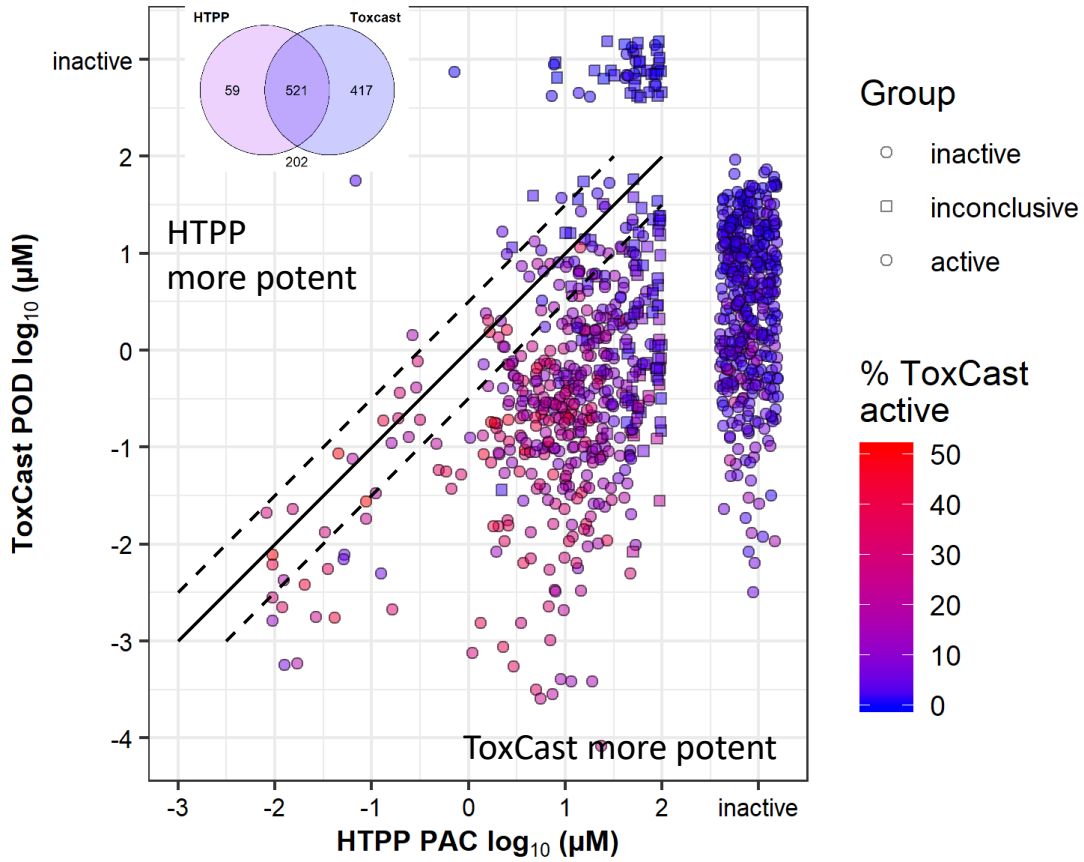
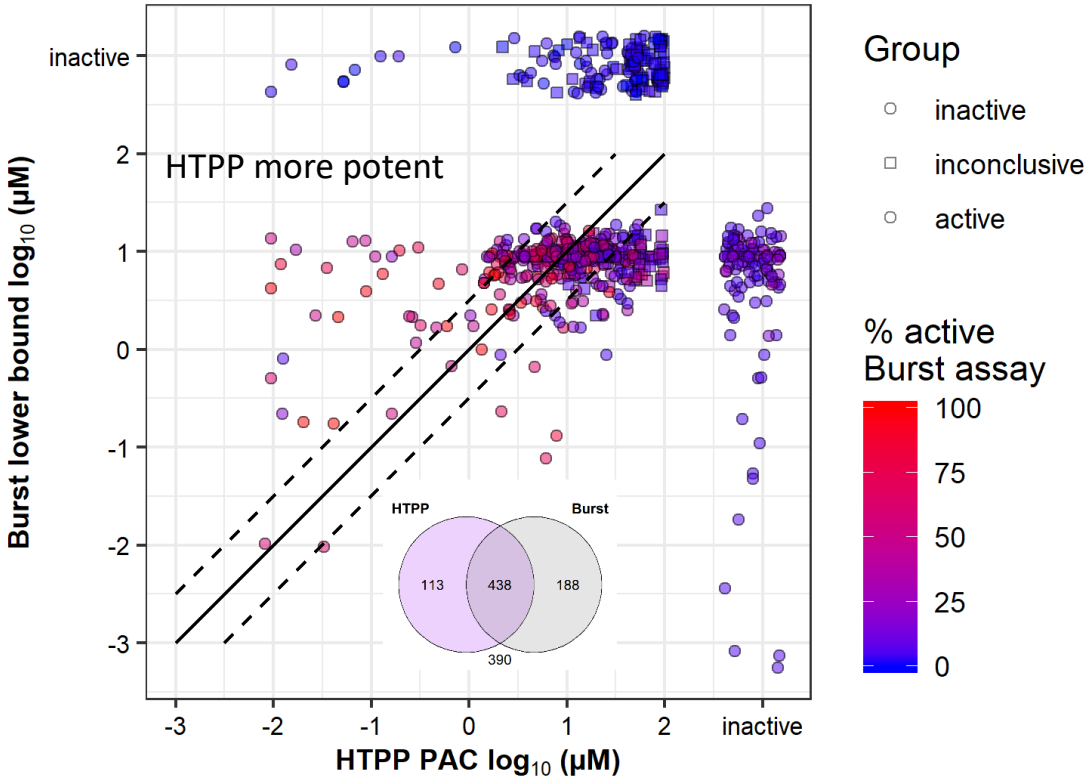
HTPP Screening Results (2)

Comparison with ToxCast screening results:





⇨ **Less potent than ToxCast POD**

⇨ **More potent than the ToxCast cytotoxicity burst estimate**

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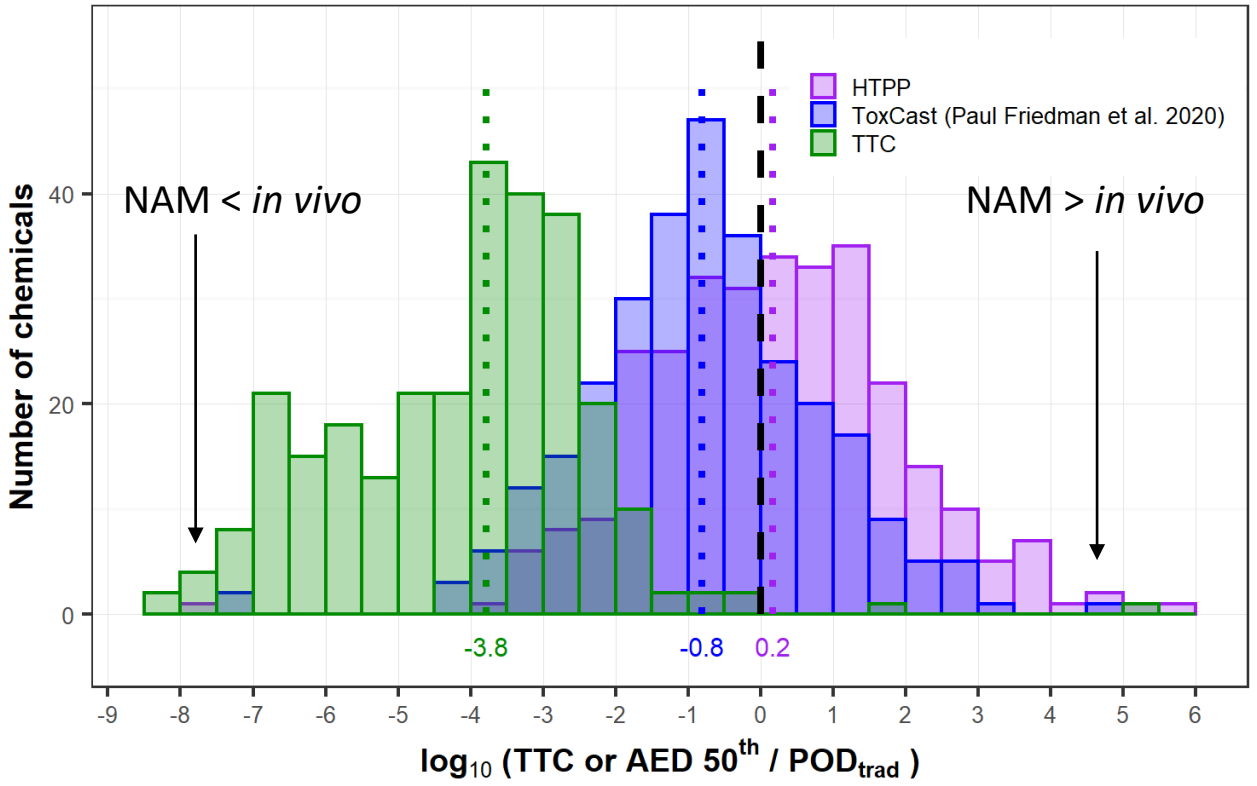
Comparison to *in vivo* Effect Values & other NAMs

- 303 chemicals were active and had pharmacokinetic (PK) information

in vitro POD

IVIVE

administered equivalent dose (AED)



⇨ **HTPP AEDs are higher than ToxCast-derived AEDs and TTC values**

⇨ **78% of HTPP AED are within 2 orders of magnitude of the *in vivo* POD**

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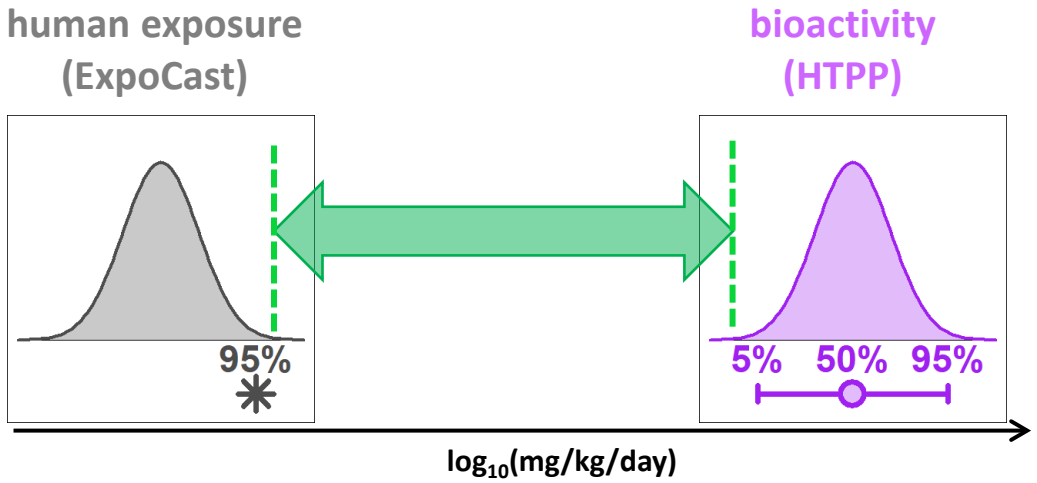
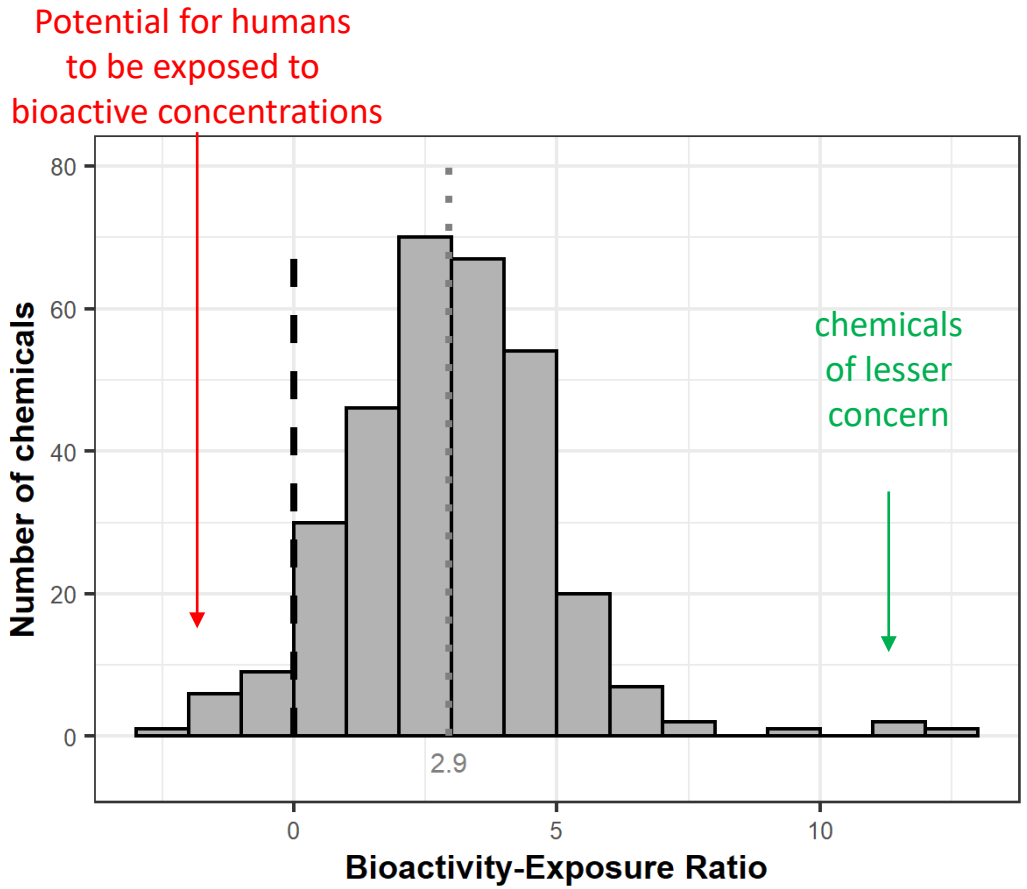
Comparison to Exposure Estimates





- for 49% of chemicals, predicted exposure is > 1000x lower than estimated bioactivity
- for a small set of chemicals, the BER was negative, indicating a potential for humans to be exposed to bioactive concentrations of these chemicals

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Preliminary results. Do not cite or quote.

Feature Selection & Profile Comparison

Feature Selection

1300 features

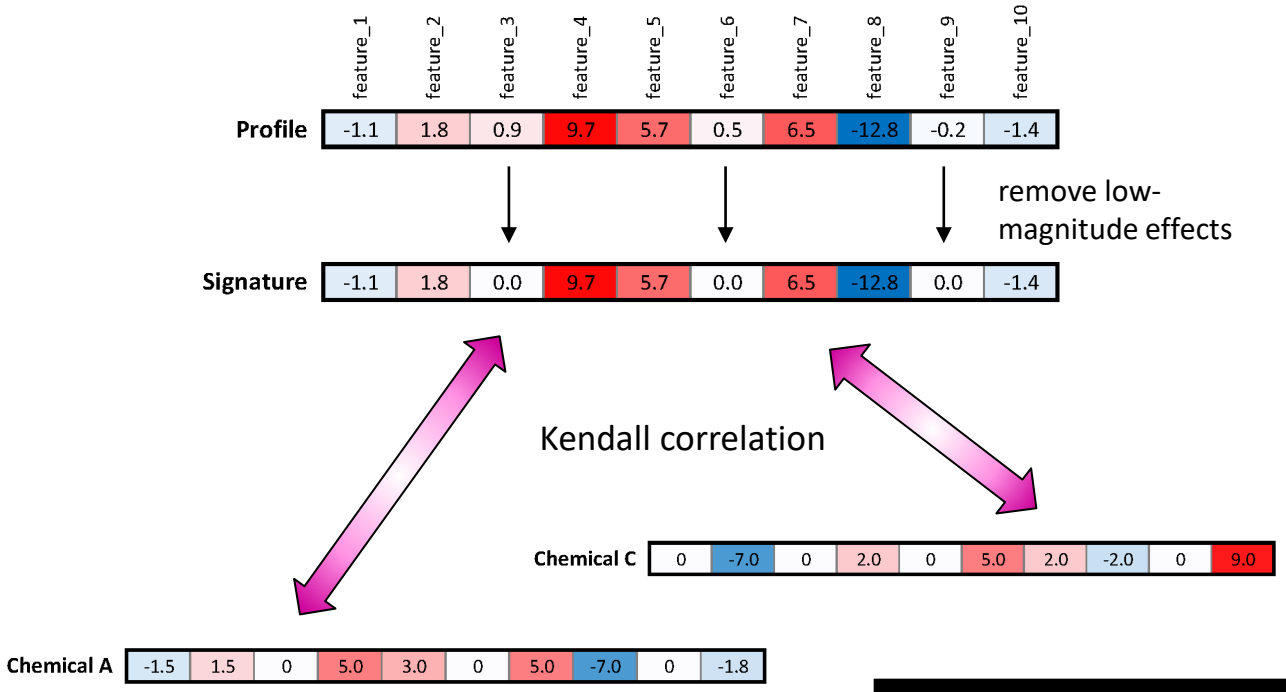
1. remove features that do not provide any information
(i.e. have 0 variance)

2. remove features that are not reproducible
(high variation between treatments of different biological replicates)

3. remove features that are highly correlated
(using recursive feature elimination)

317 features

Profile Comparison



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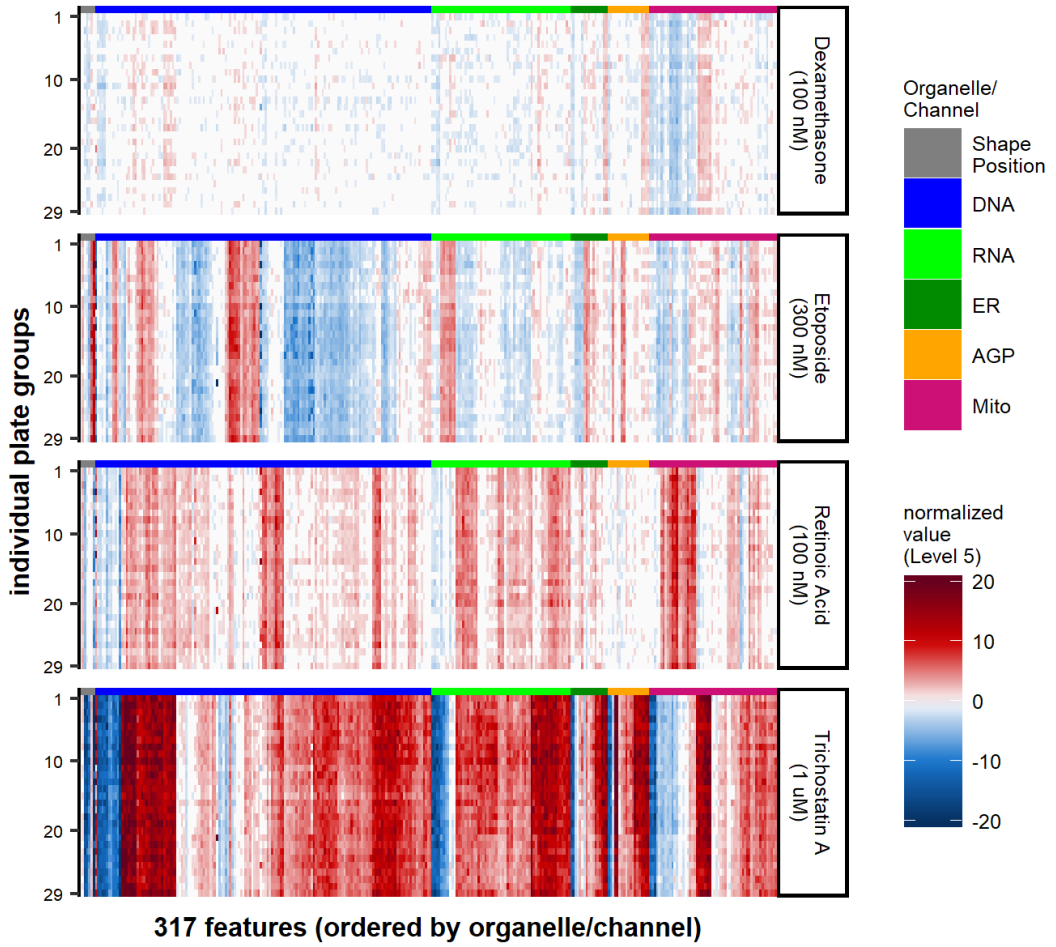
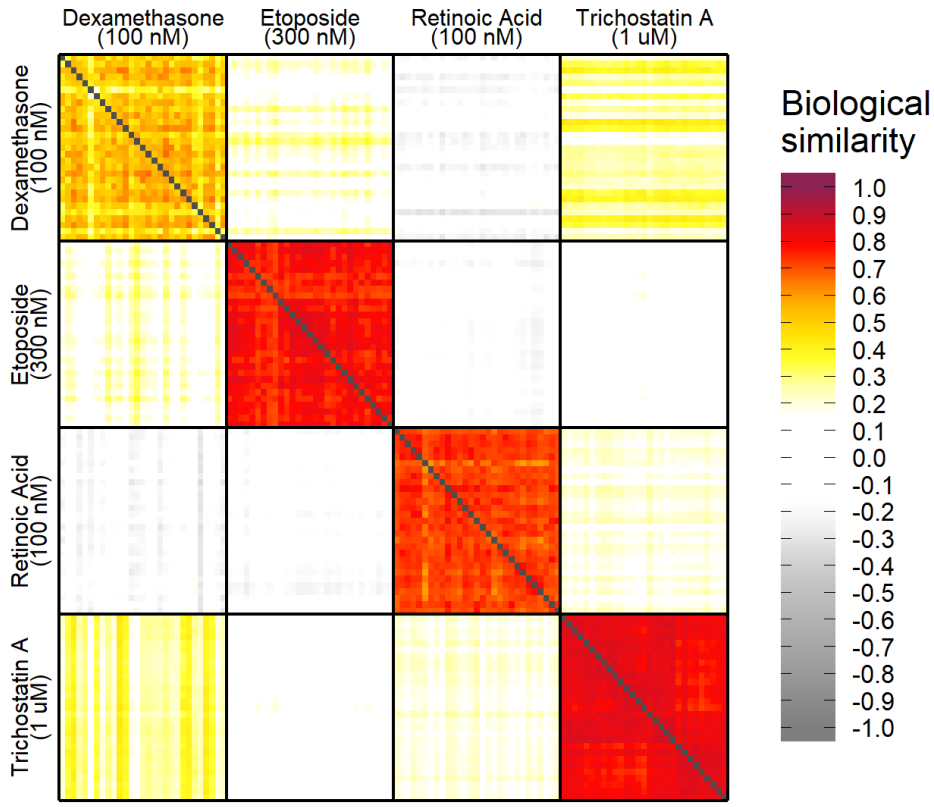
Reproducibility: Phenotypic Profiles





⇨ **Phenotypic profiles are highly reproducible across different plates**

Hypothesis: Chemicals with similar mechanisms will display similar profiles.

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Example: Nuclear Receptor Modulators (I)

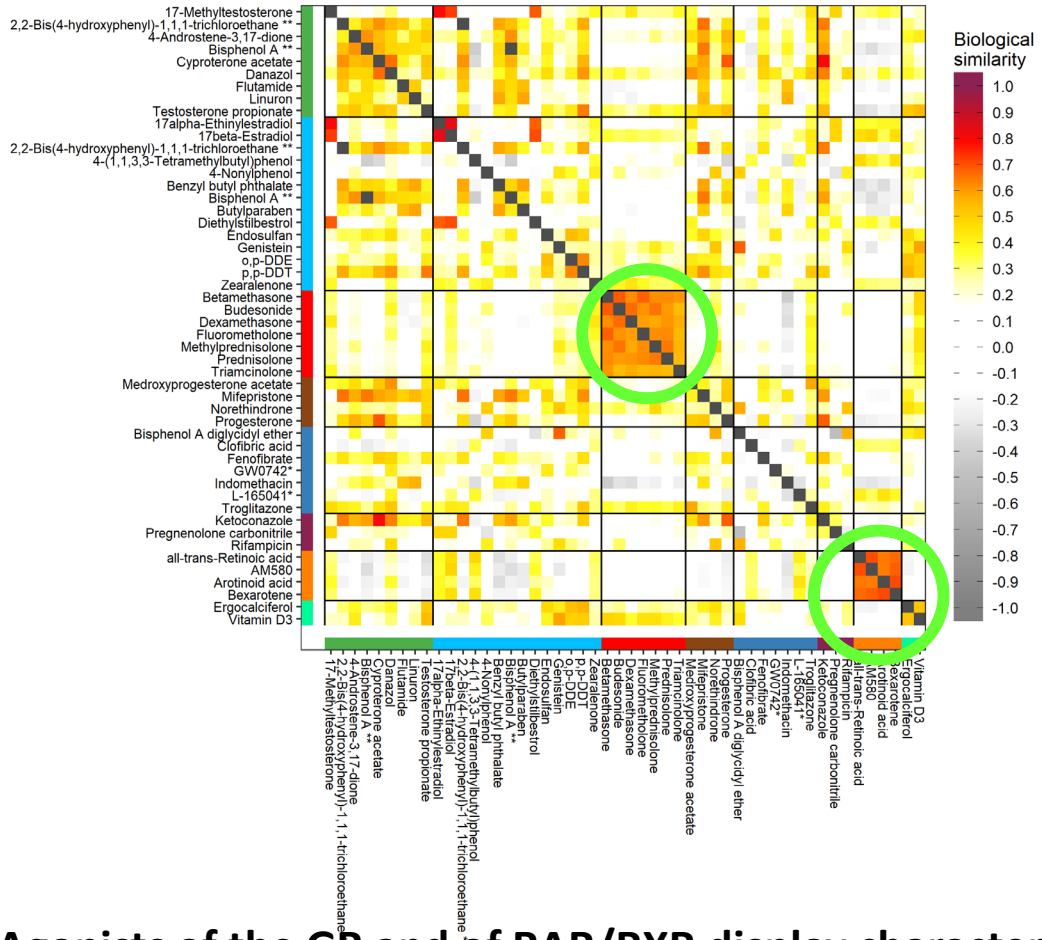
- 52 chemicals were annotated as targeting a nuclear receptor

Preliminary results. Do not cite or quote.

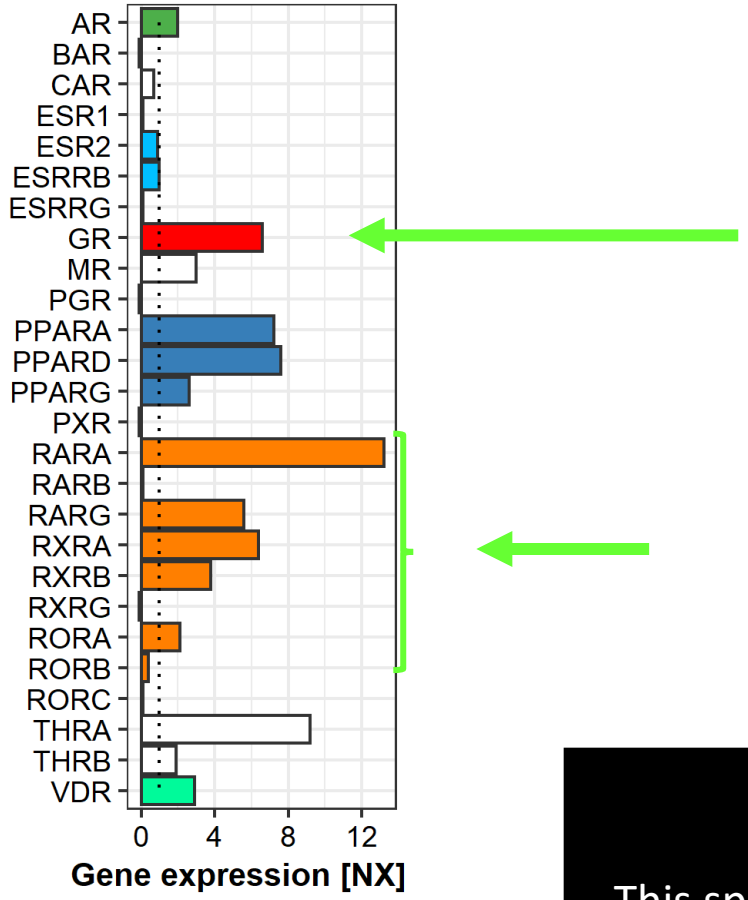
target

AR
ESR
GR
PGR
PPAR
PXR
RAR
VDR

Biological similarity in HTPP



Gene expression in U-2 OS



⇨ **Agonists of the GR and of RAR/RXR display characteristic profiles**

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

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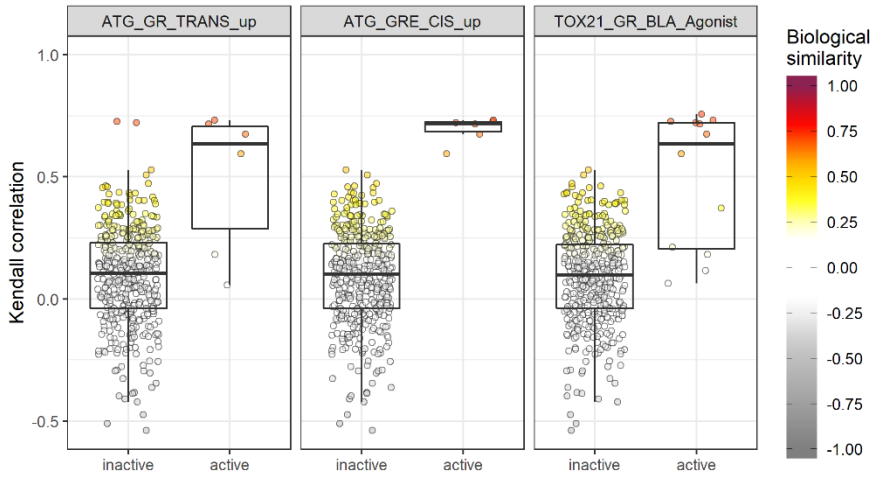
Example: Nuclear Receptor Modulators (II)

target

- AR
- ESR
- GR
- PGR
- PPAR
- PXR
- RAR
- VDR

Biological similarity in HTPP



Chemicals with similar profiles to dexamethasone tend to be active in **ToxCast GR assays**

Chemicals with similar profiles to all-trans retinoic acid tend to be active in **ToxCast RAR / RXR assays**

⇨ **Certain molecular mechanisms result in characteristic phenotypic profiles**

Preliminary results. Do not cite or quote.

This space reserved for video image.

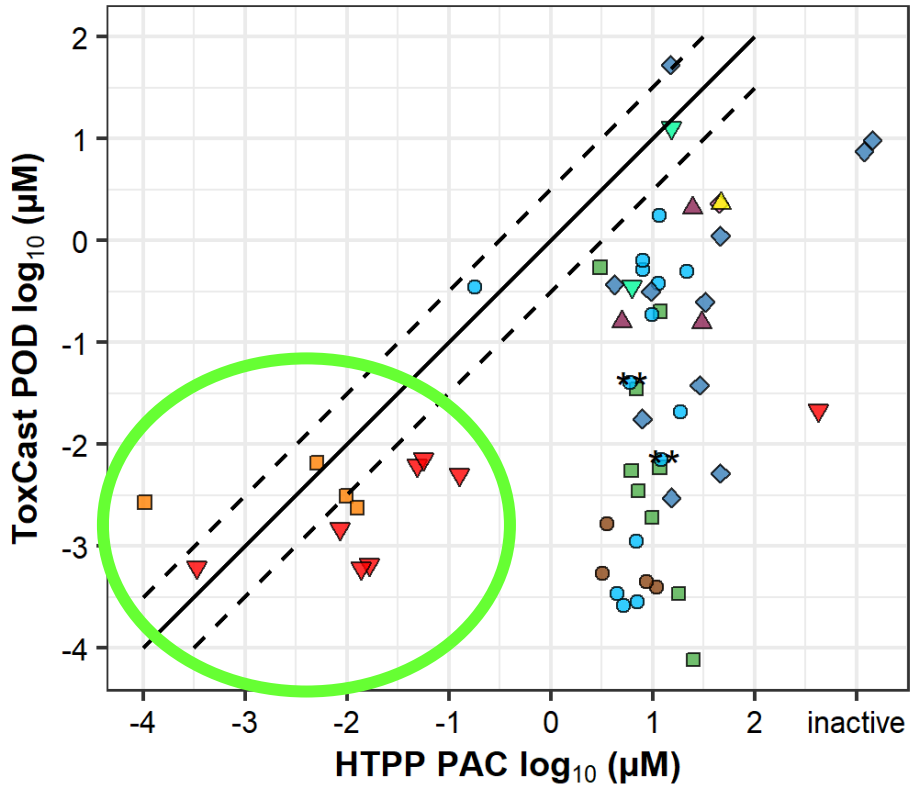
Example: Nuclear Receptor Modulators (III)

Preliminary results. Do not cite or quote.

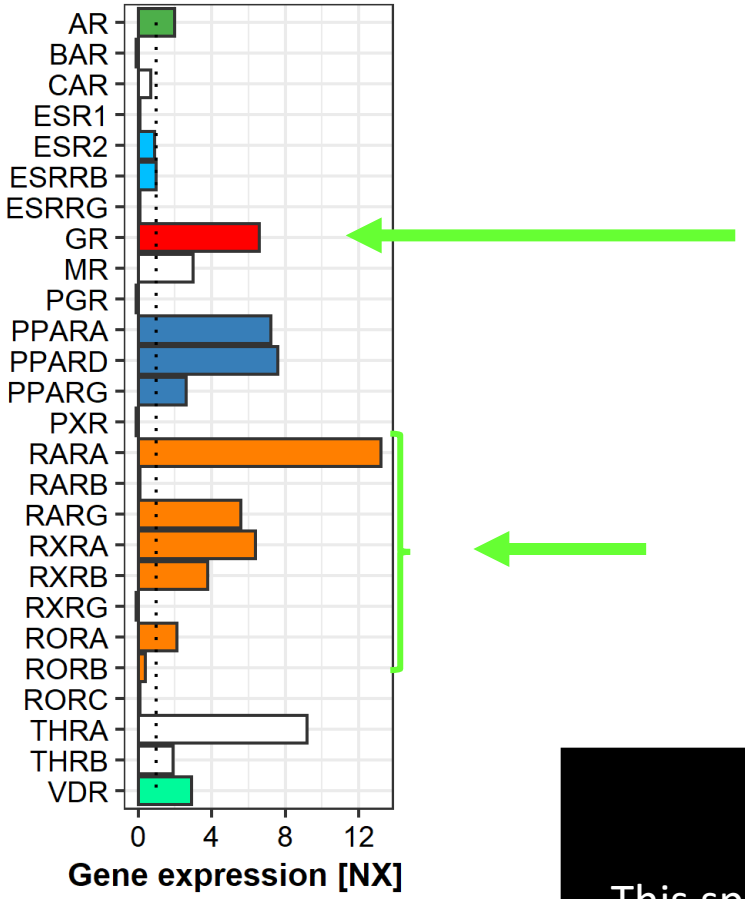
target

- AR
- ESR
- GR
- PGR
- PPAR
- PXR
- RAR
- VDR

Comparison to ToxCast potencies



Gene expression in U-2 OS



- ⇨ **For two receptor systems that are expressed (GR, RAR/RXR) potencies were comparable with ToxCast**
- ⇨ **For all other receptors, we are much less sensitive than ToxCast (off-target effects?)**

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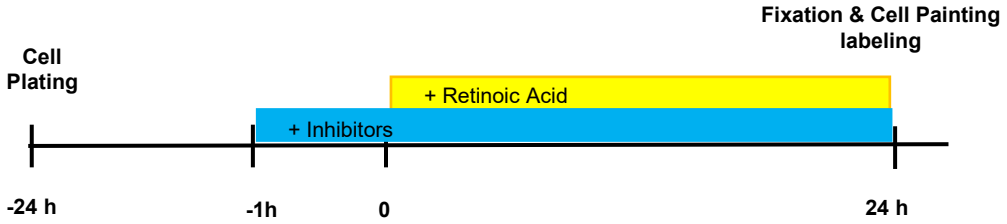
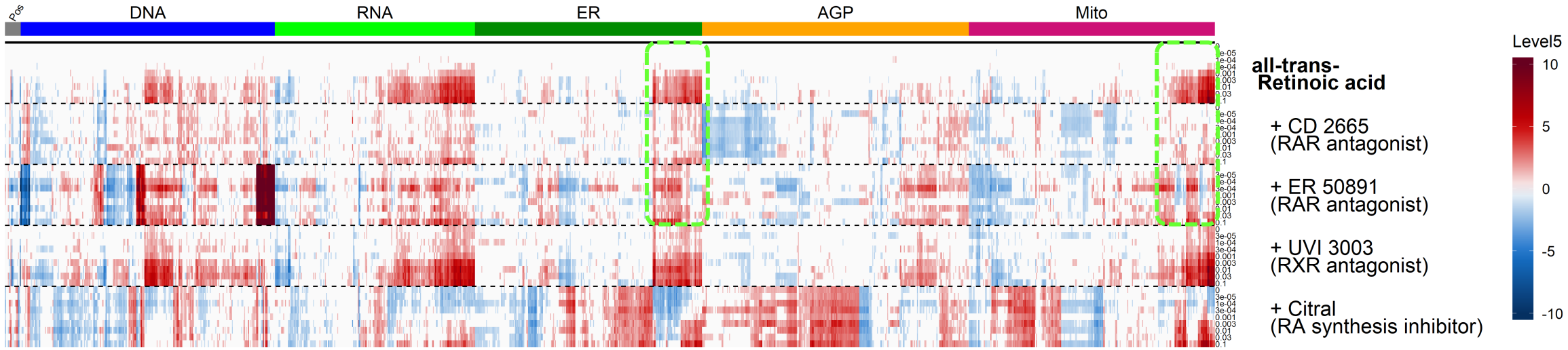
Pharmacological Blockade of Phenotypic Effects





⇨ **RAR but not RXR antagonists block the retinoid phenotype**

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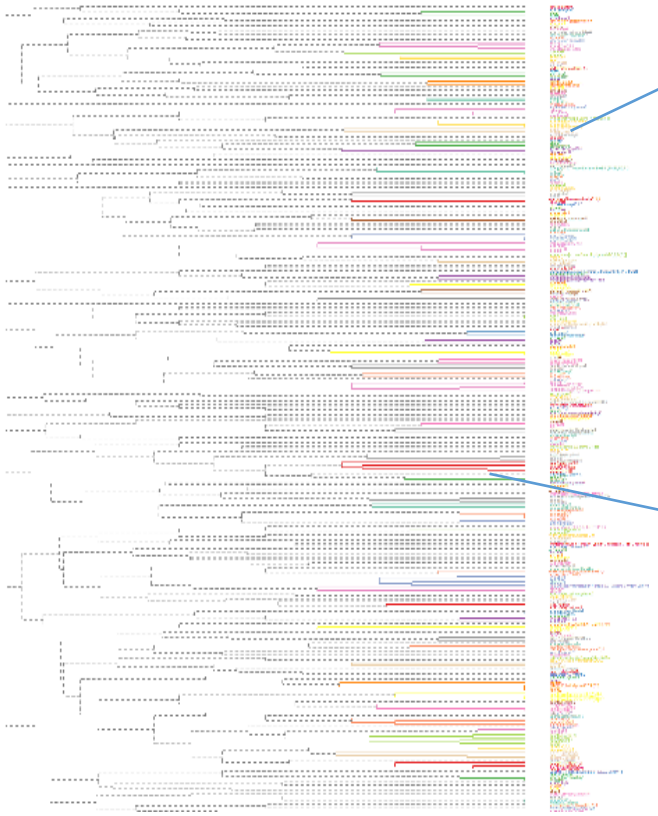
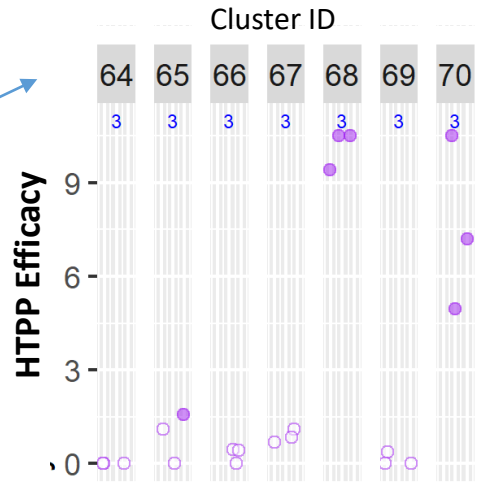
Preliminary results. Do not cite or quote.

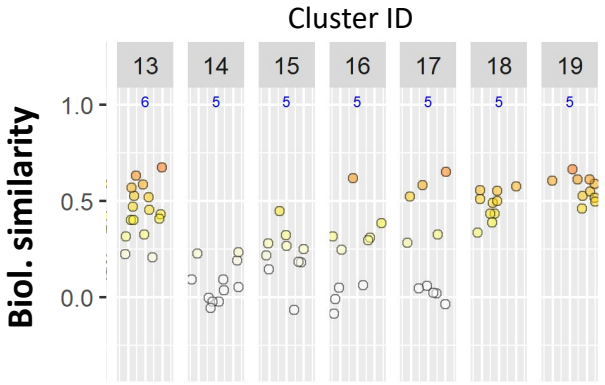
Structural Similarity Translates to Biological Similarity

Dendrogram using structural information (ToxPrints) → 680 clusters





⇨ **Structurally similar chemicals tend to display similar efficacies**



⇨ **Structurally similar chemicals tend to display similar phenotypes**

⇨ **Structurally similar chemicals tend to be biologically similar**

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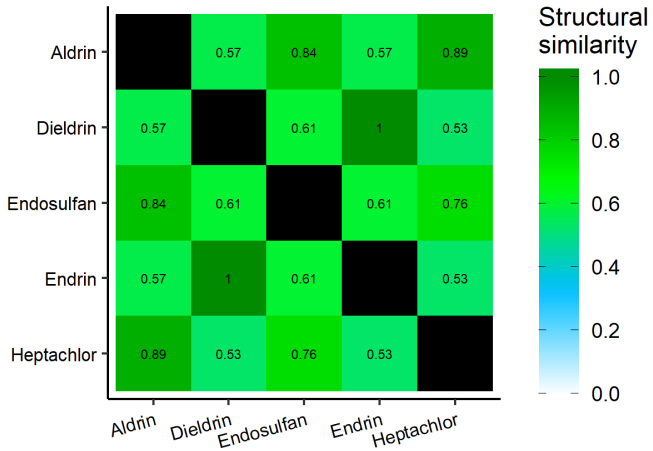
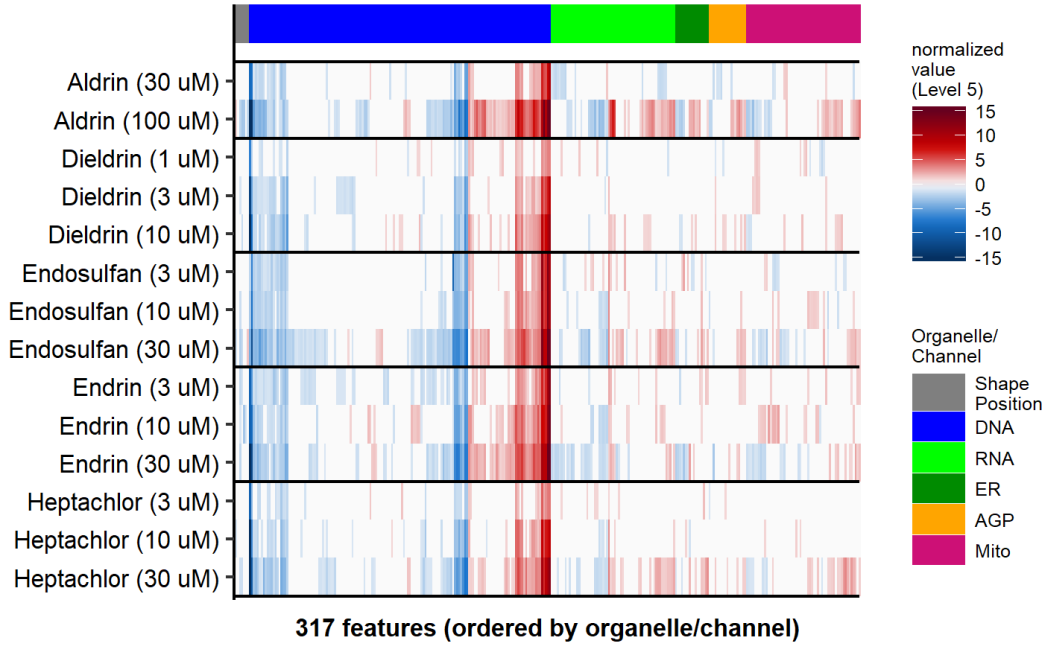
Preliminary results. Do not cite or quote.

Application to Environmental Chemicals:
Example: Organochlorines

structural similarity

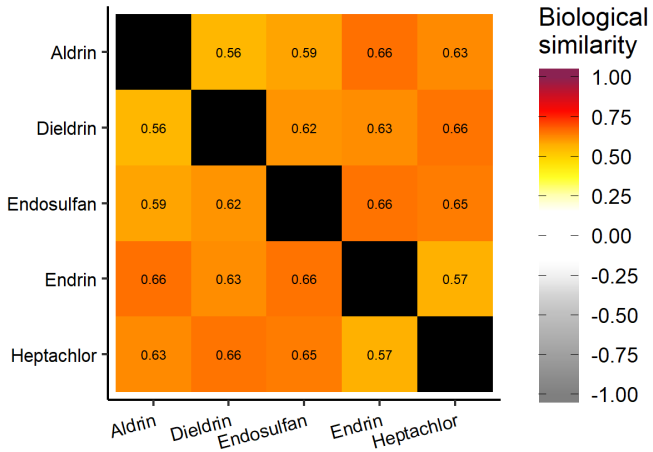
(Jaccard similarity, ToxPrints)





biological similarity

(Kendall similarity)



⇨ **Organochlorines are structurally and phenotypically similar**

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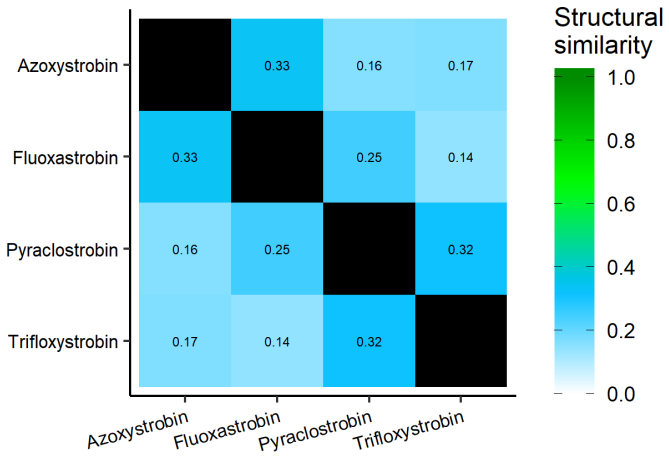
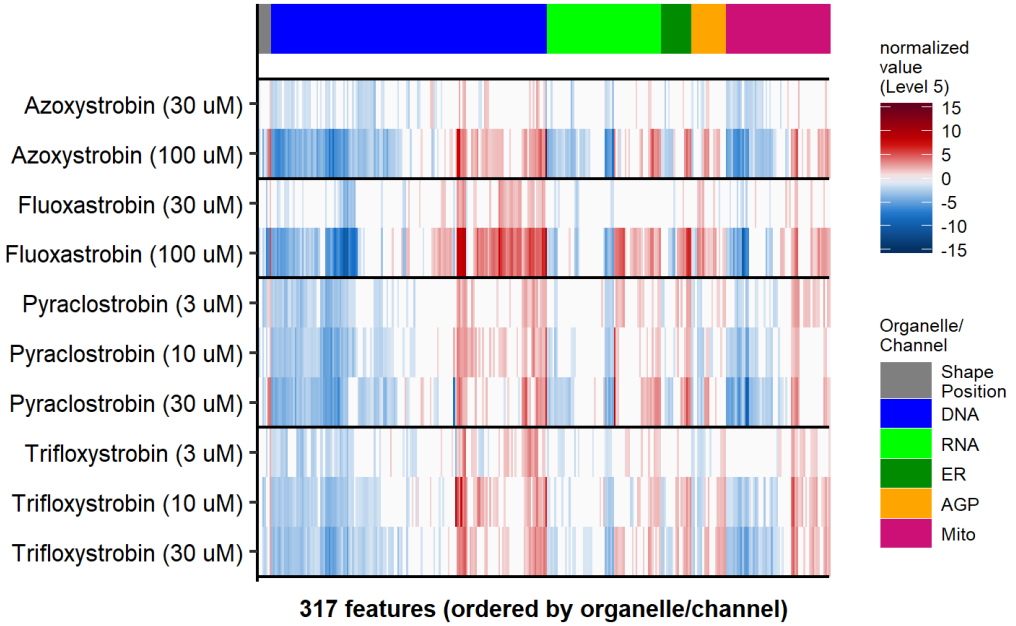
Preliminary results. Do not cite or quote.

Application to Environmental Chemicals:
Example: Strobilurins

structural similarity

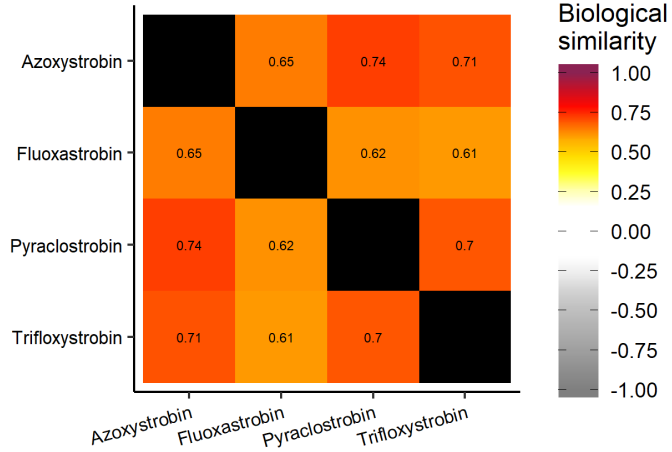
(Jaccard similarity, ToxPrints)





biological similarity

(Kendall similarity)



⇨ **Strobilurins have less structural similarity, yet share the same molecular target and produce similar phenotypes**

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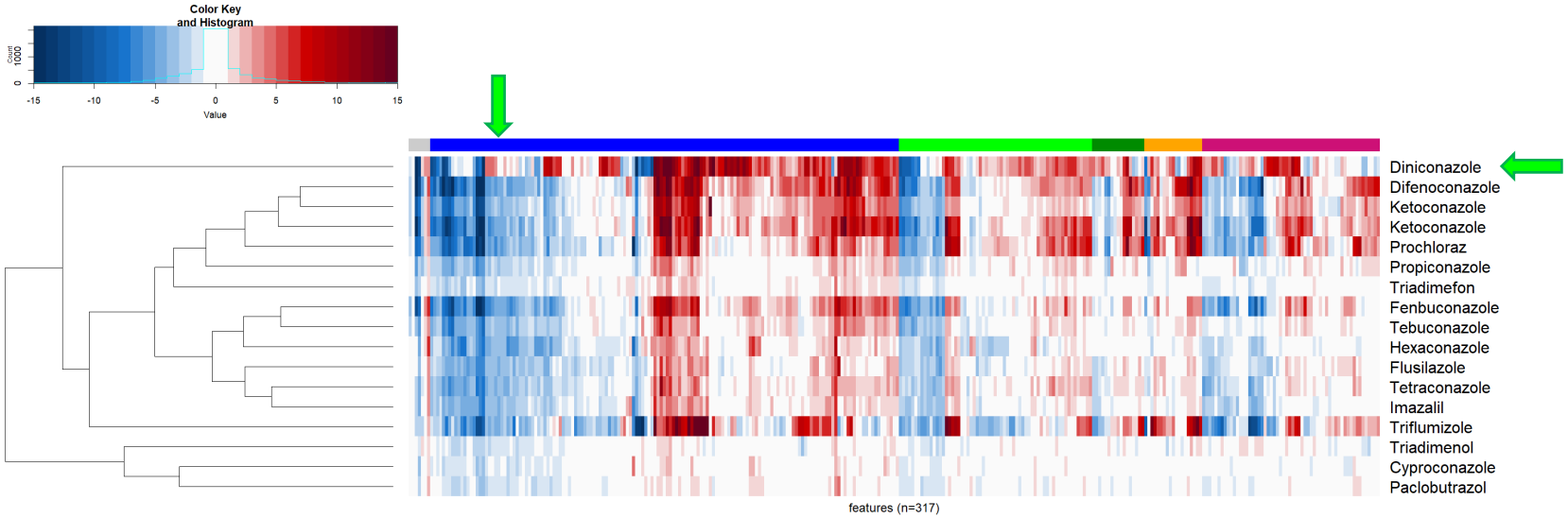
Preliminary results. Do not cite or quote.

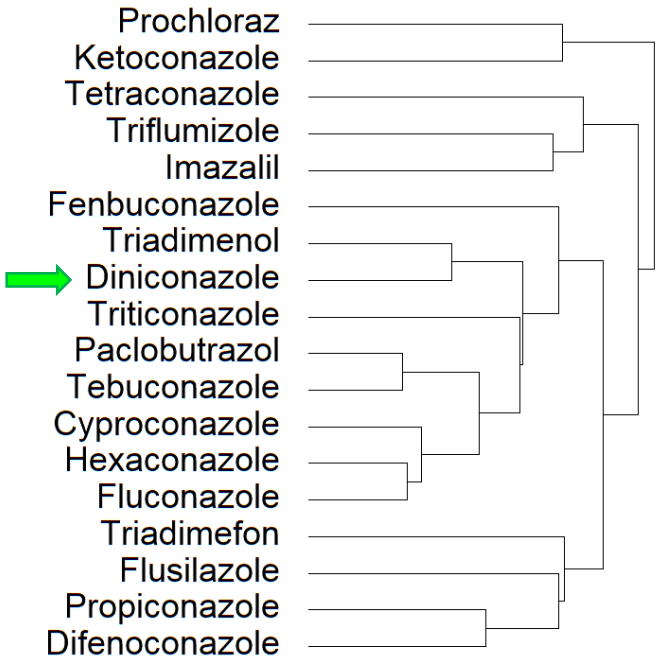
Application to Environmental Chemicals:
Example: Conazoles

- group of fungicides
- disturb ergosterol synthesis via CYP51 and CYP61 (target absent in mammals)

biological similarity



structural similarity
(based on ToxPrints)



⇨ **most conazoles are phenotypically similar**

⇨ **Diniconazole is phenotypically different from the other active conazoles**

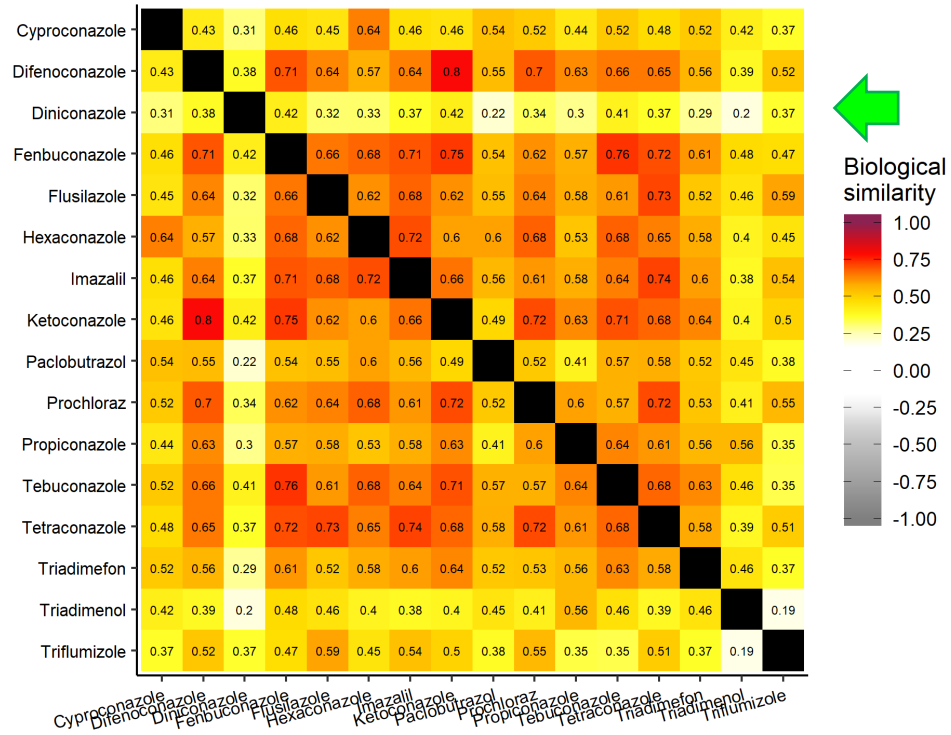
This space reserved for video image.

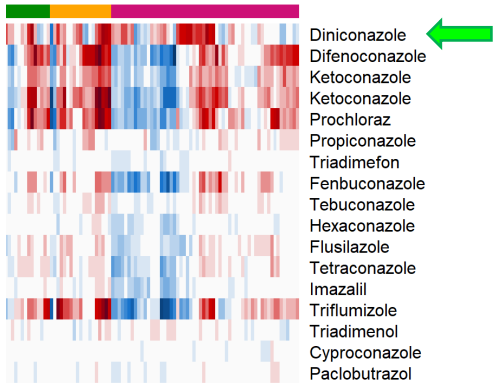
Preliminary results. Do not cite or quote.

Application to Environmental Chemicals:
Example: Conazoles

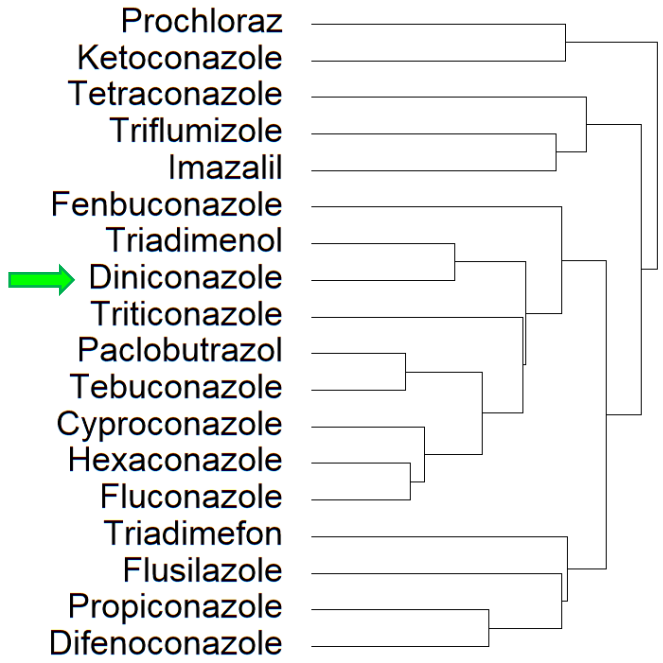
- group of fungicides
- disturb ergosterol synthesis via CYP51 and CYP61 (target absent in mammals)

biological similarity





structural similarity
(based on ToxPrints)



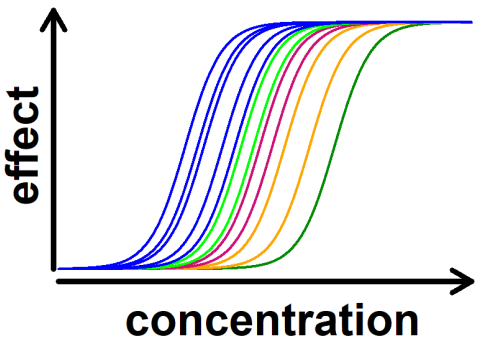
⇨ **most conazoles are phenotypically similar**

⇨ **Diniconazole is phenotypically different from the other active conazoles**

Preliminary results. Do not cite or quote.

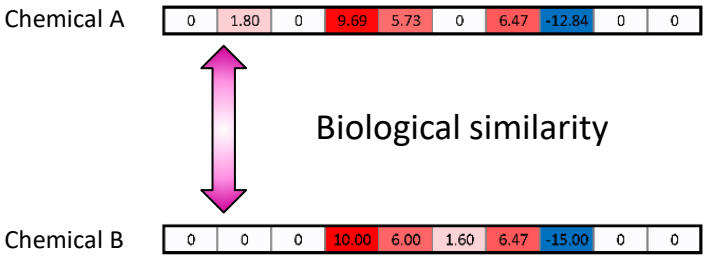
This space reserved for video image.

Conclusions



Application 1: Potency estimation

- HTPP can be used to derive *in vitro* potency estimates
- These *in vitro* potency estimates are often comparable or more conservative than *in vivo* PODs



Application 2: Mechanistic prediction

- Structural similarity → biological similarity
- Similar mechanisms → biological similarity

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Outlook

- **Combine HTPP with HTTr**
  - compare results, both in terms of potencies and mechanisms
  - increased potential to discern molecular mechanisms

SOT Presentation by Dr. Joshua Harrill
"In Vitro Molecular Points-of-Departure (PODs) from High-Throughput Profiling Assays"
Tuesday, March 23, 2021
11:15 AM-2:00 PM US Eastern Time

- **Expand Coverage of Biological Space**
  - deploy assay across diverse cell lines that express different receptors/pathways
  - proof-of-concept (Gustafsdottir *et al.* 2013, Willis *et al.* 2020)
  - expansion to other species

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