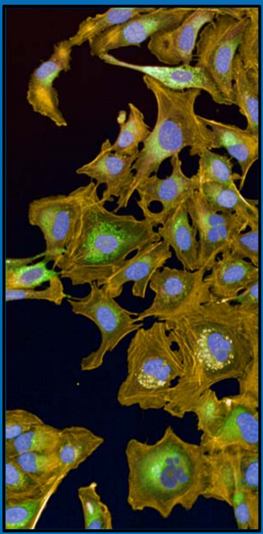

# Application of Cell Painting for chemical hazard evaluation in support of screening-level assessments

**Jo Nyffeler, PhD**

Helmholtz Centre for Environmental Research (UFZ)
Leipzig, Germany

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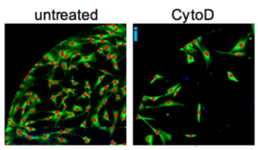
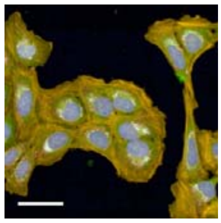
Jo.Nyffeler@ufz.de

**Webinar for ASCCT & ESTIV**
**November 27th, 2023**

## Introduction: Dr. Jo 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 imaging-based profiling ('Cell Painting'), computational toxicology
- **Group leader at Helmholtz Centre for Environmental Research (UFZ), Leipzig, Germany**
  - High-throughput methods for ecotoxicology

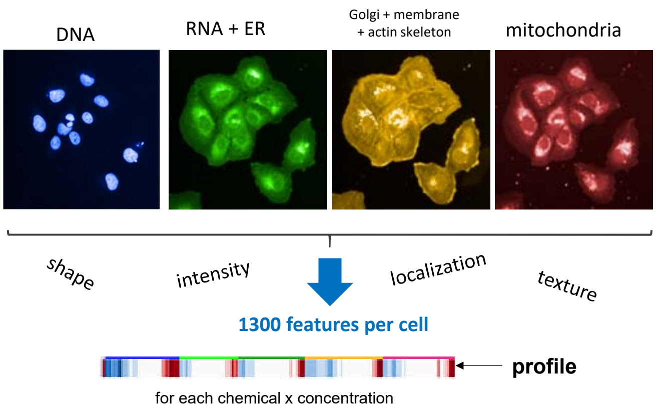


2

## 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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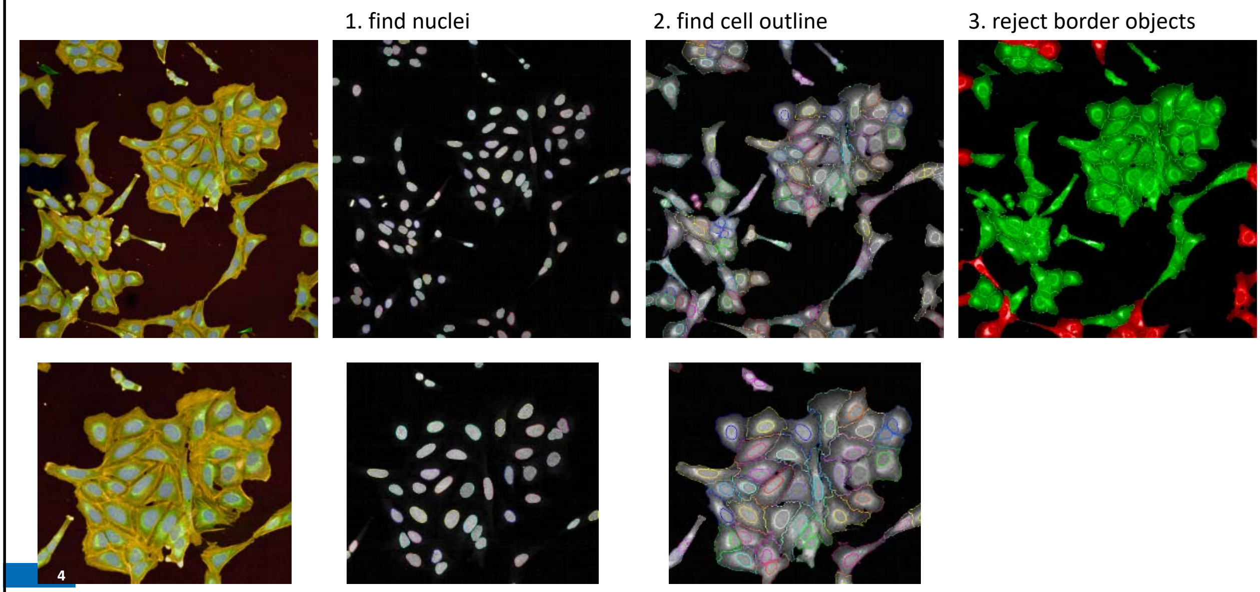
## Image Analysis Workflow → Image Segmentation

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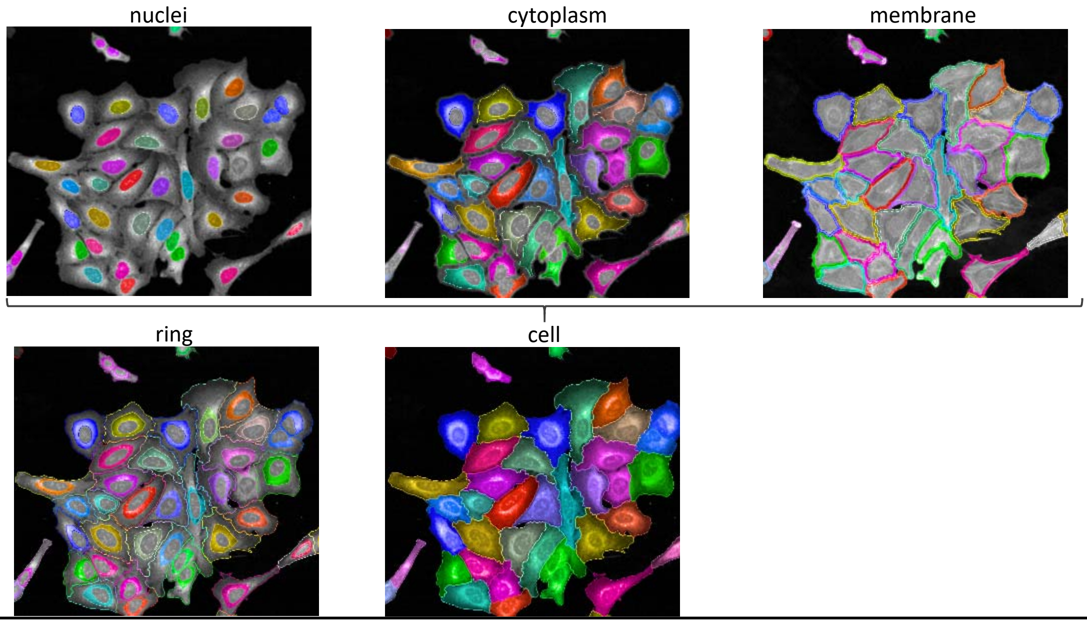
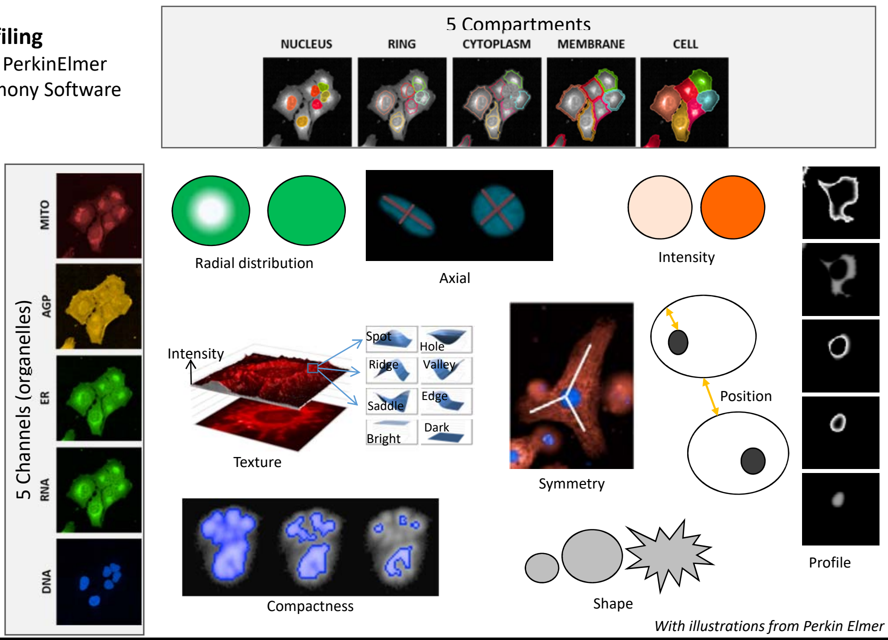
## Define Cellular Compartments






## What is a "feature"?

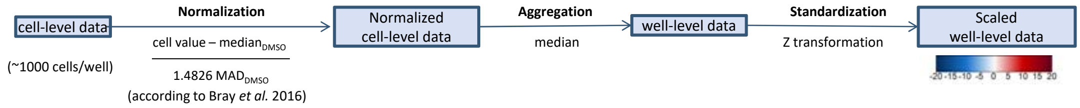
**Profiling**
with PerkinElmer
Harmony Software

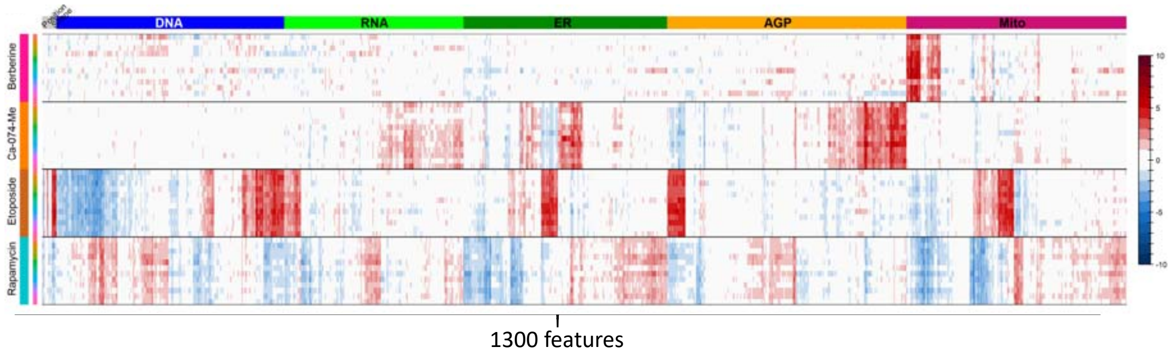



= 1300 features

*With illustrations from Perkin Elmer*

## Example Chemicals: Quantitative Observation



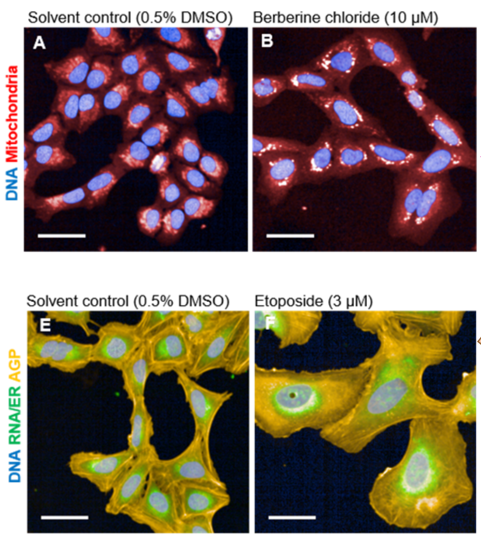




⇨ **Qualitative observations can be quantified**

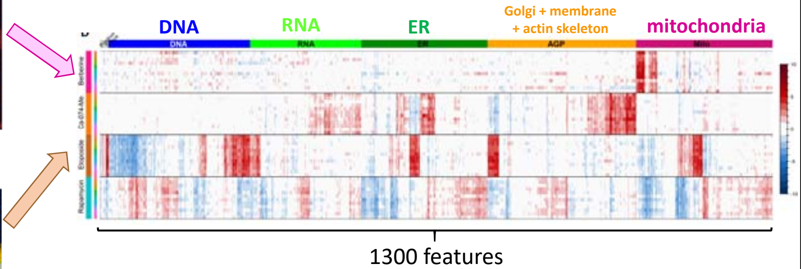


adapted from Nyffeler *et al.* 2020

## Exemplary Chemicals




→ Mitochondrial compactness/texture




→ Cells are larger

⇨ **Visual phenotypic changes can be represented quantitatively**



adapted from Nyffeler *et al.* 2020

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Application of Cell Painting for chemical hazard evaluation in support of screening-level chemical assessments

Jo Nyffeler [a,b], Clinton Willis [a], Felix R. Harris [a,c], M.J. Foster [a,c], Bryant Chambers [a], Megan Culbreth [a], Richard E. Brockway [a,c], Sarah Davidson-Fritz [a], Daniel Dawson [a], Imran Shah [a], Katie Paul Friedman [a], Dan Chang [a], Logan J. Everett [a], John F. Wambaugh [a], Grace Patlewicz [a], Joshua A. Harrill [a,*]

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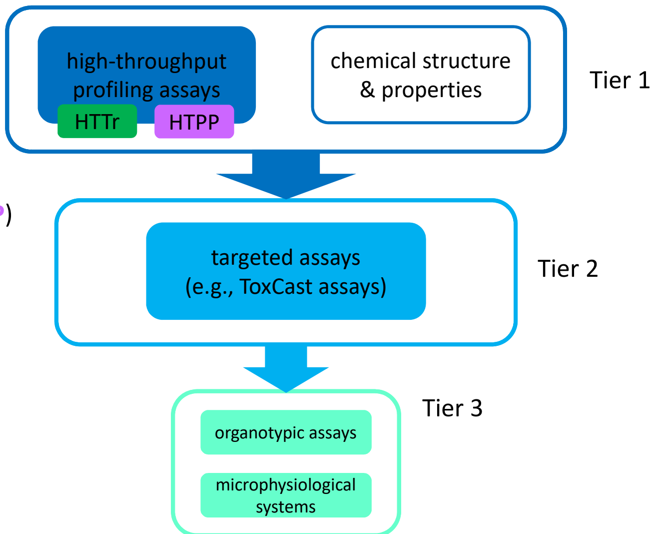
# USEPA's Tiered Hazard Evaluation Strategy based on New Approach Methods (NAMs)

**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)

**Focus**

- **Prioritization: False positives are preferred over false negatives**




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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# Challenges of Environmental Chemicals

- Often low expected bioactivity
- Often lack a specific molecular target in human-based cell models
- 'poly-pharmacology'
- Responses can be associated with general cell stress

⇨ **more challenging for hit identification than drug-like chemicals**

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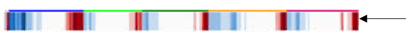
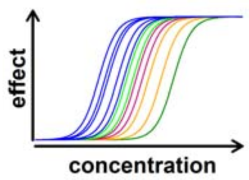
# Two Applications

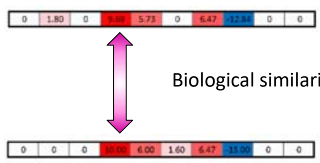



**Application 1**

concentration-response modelling




**Application 2**




**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
- Nyffeler *et al.* (2022). Toxicol Appl Pharmacol. PMID: 35483669

**Compare profiles with annotated reference chemicals**
**→ putative mechanisms**

- Nyffeler *et al.* (2022). Toxicol Appl Pharmacol. PMID: 35483669
- Nyffeler *et al.* (2023). Toxicol Appl Pharmacol. PMID: 37044265

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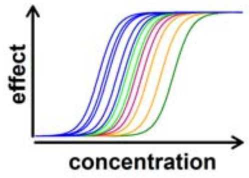
**Application 1**

concentration-response modelling

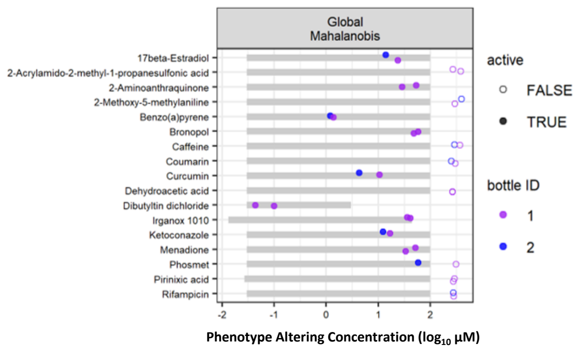


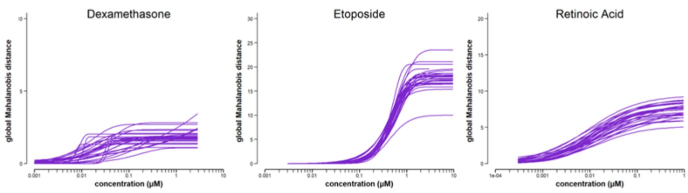

# **Application 1:** Potency Estimation



## The „U-2 OS Toxcast“ screen

| „U-2 OS Toxcast“ screen | |
|---|---|
| Cell line | U-2 OS (human osteosarcoma) |
| Exposure duration | 24 h |
| # Chemicals | 1199 unique chemicals |
| # concentrations | 8 |
| # biological replicates | 4 |
| # technical replicates | 1 |

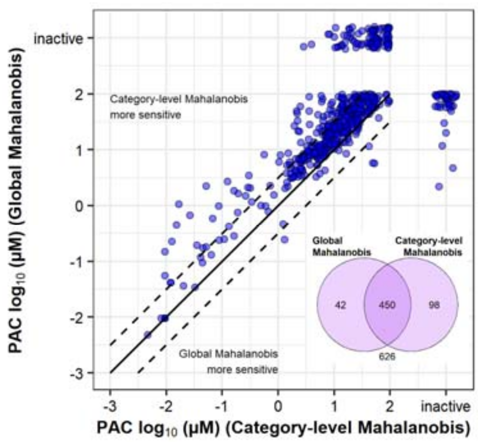


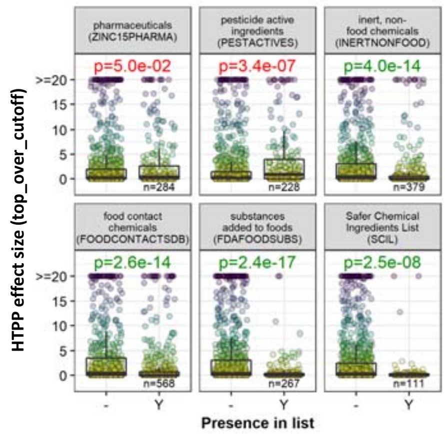



⇨ **Assay performance is monitored**

## Overview of the results




Are active chemicals overrepresented in certain lists?




- ⇨ 49% (590/1196) of chemicals were active with at least one approach.
- ⇨ Active chemicals are overrepresented in pharmaceutical and pesticide lists
- ⇨ Inactive chemicals are overrepresented in food contact chemicals

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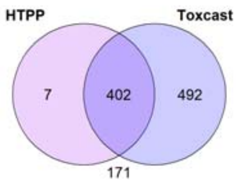
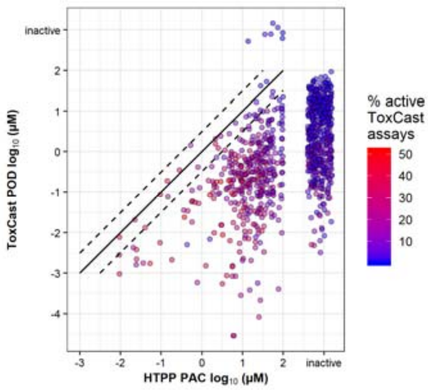
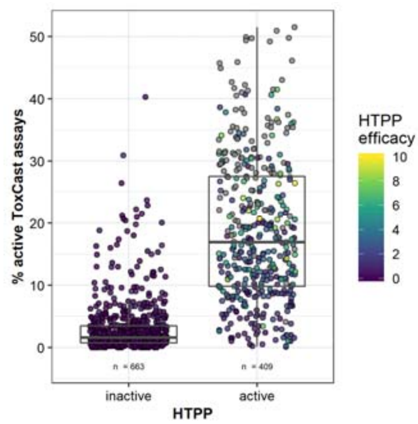
## How does HTPP compare to targeted assays (i.e., ToxCast assay battery)




- ⇨ Toxcast detects more chemicals as active
- ⇨ HTPP more sensitive than simple cytotox burst assays
- ⇨ Chemicals active in HTPP → active in many ToxCast assays

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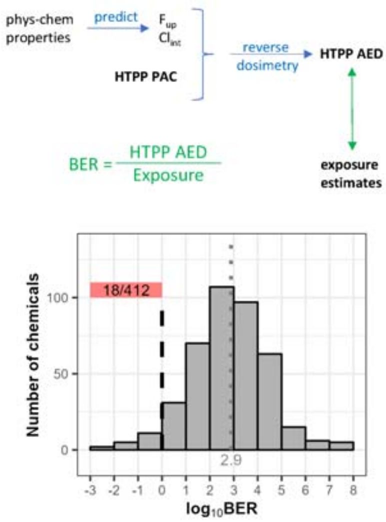
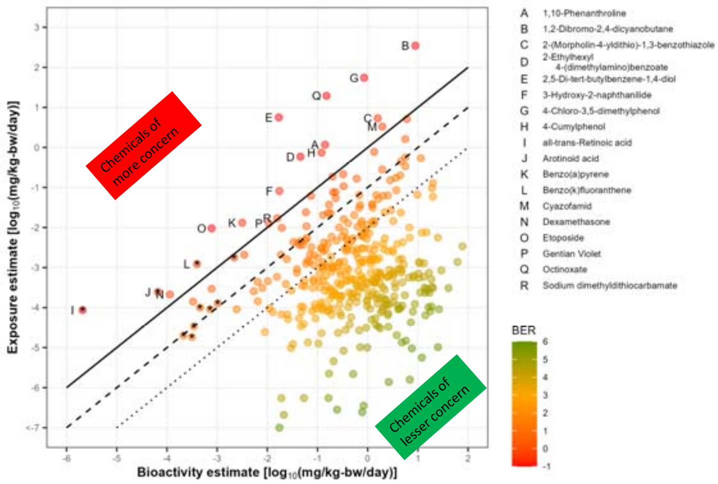
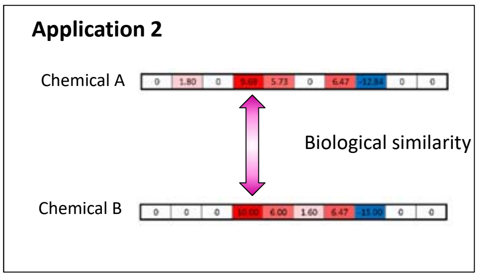
## Bioactivity-Exposure Ratio for prioritizing chemicals




⇨ **HTPP can help to prioritize chemicals**






# Application 2:
# Mechanistic Prediction

# Feature Selection & Profile Comparison

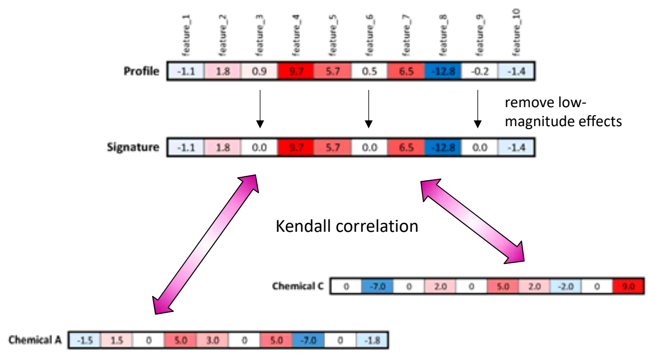
## Feature Selection

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)

289 features

## Profile Comparison

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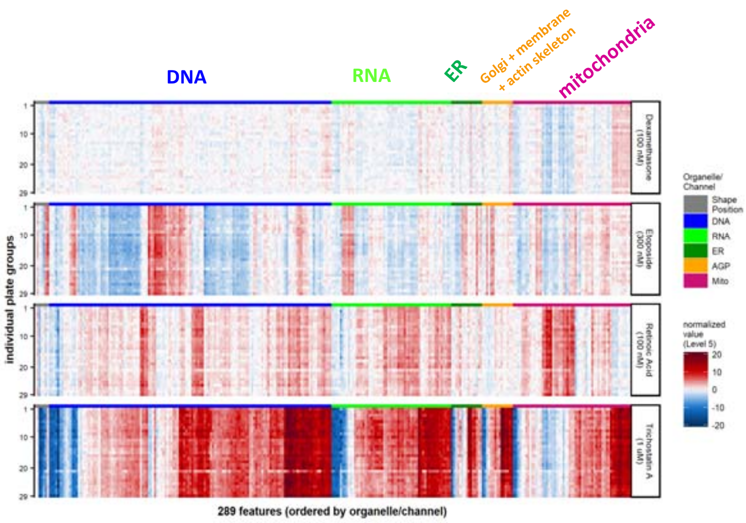
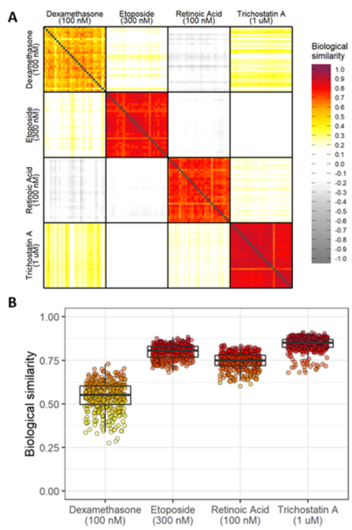
# Reproducibility of phenotypic profiles throughout the screen




⇨ **Even subtle phenotypes (Dexamethasone) are reproducible**

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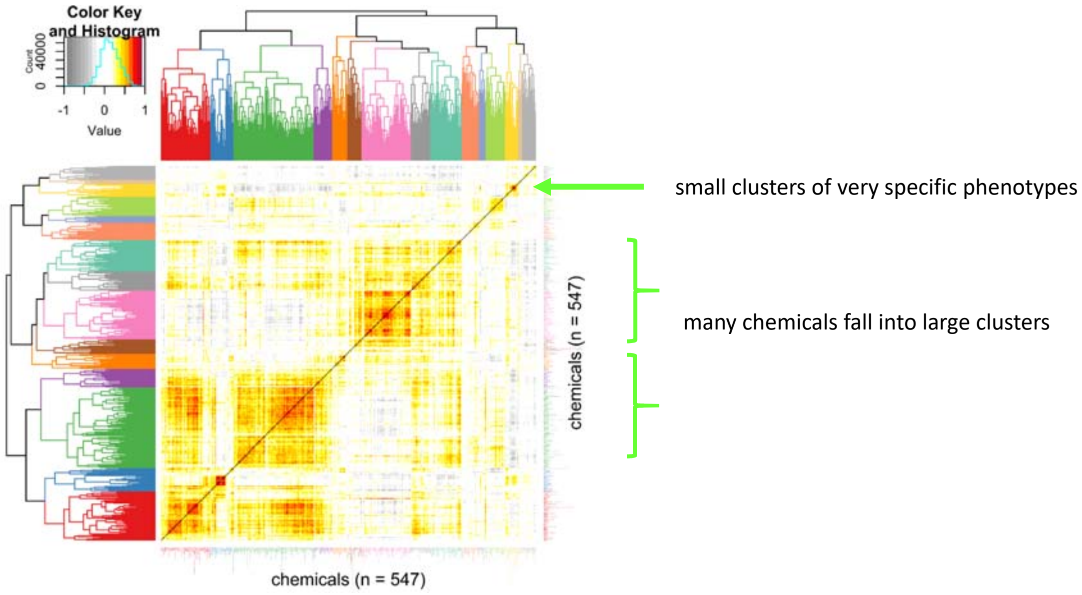
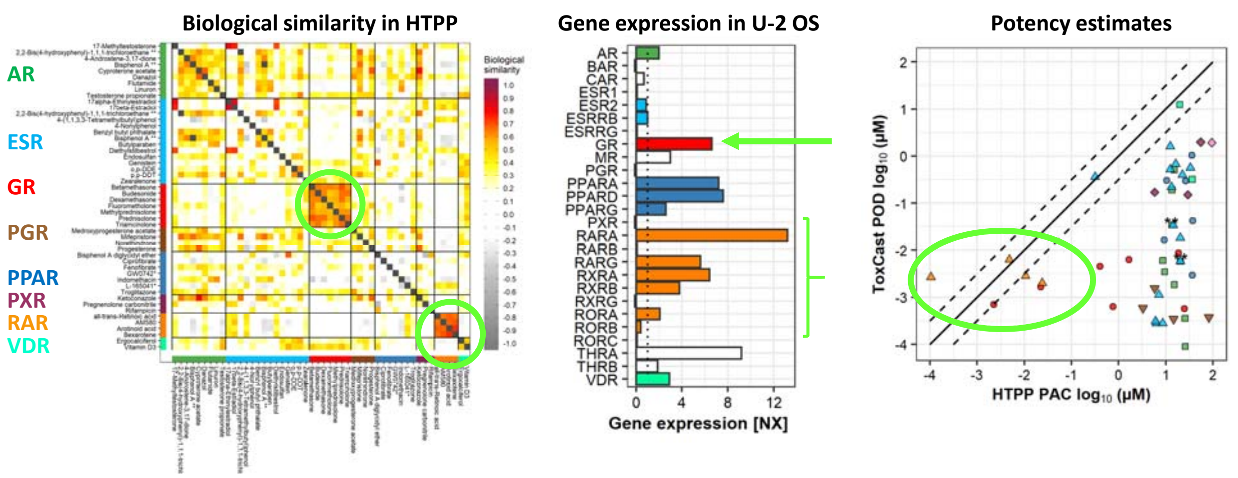
## Phenotypic similarity of all (active) chemicals






## Example: Nuclear Receptor Modulators

- 52 chemicals were annotated as targeting a nuclear receptor → 50 chemicals were active in HTPP




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

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

⇨ **For two receptor systems that are expressed (GR, RAR/RXR) potencies were comparable with ToxCast**

# Mode-of-action identification:

## Example of glucocorticoid-like chemicals

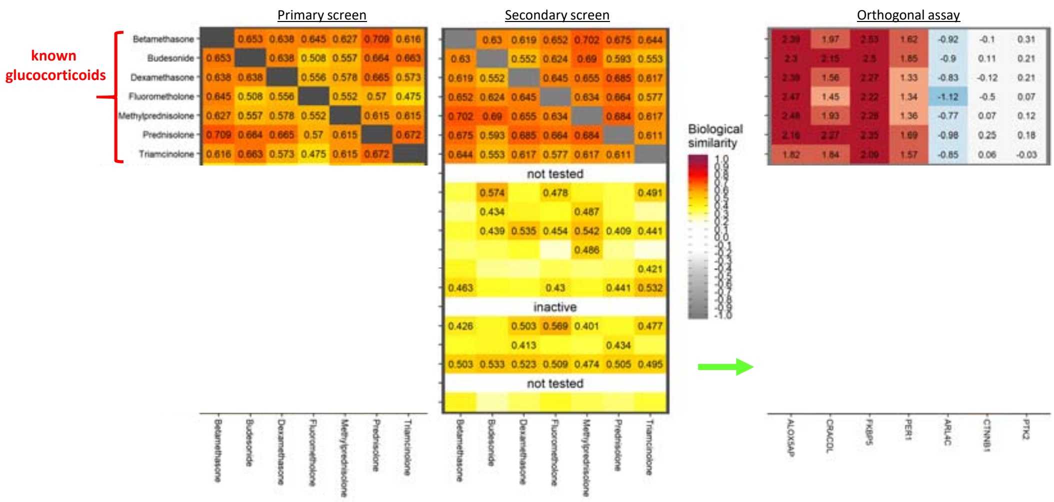
1199 chemicals

Phenotypic similarity

~ 12 candidates

Orthogonal assay: qPCR

1 chemical confirmed

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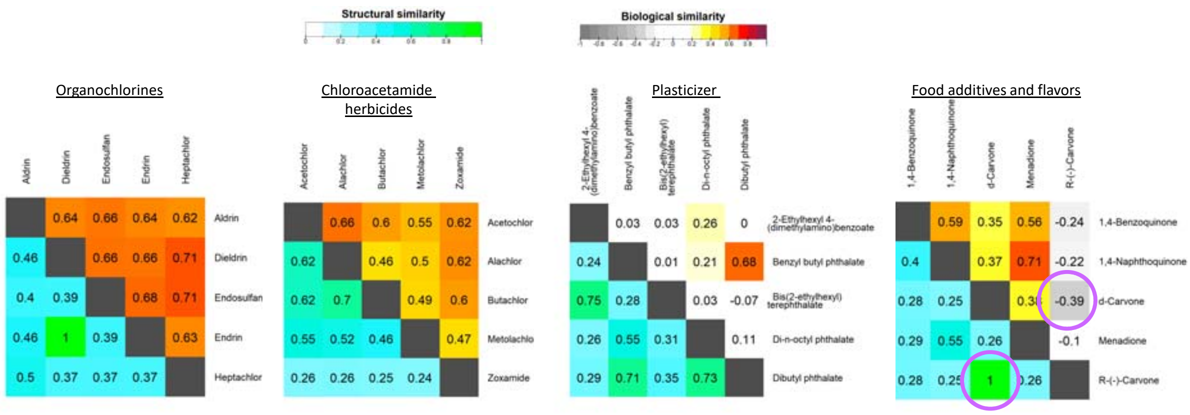
⇨ **Pyrene upregulated the same genes than known glucocorticoids.**

# What about non-drug-like chemicals?

**Structurally similar chemicals tend to induce similar phenotypes….**




**… but HTPP can also detect some differences in bioactivity!**

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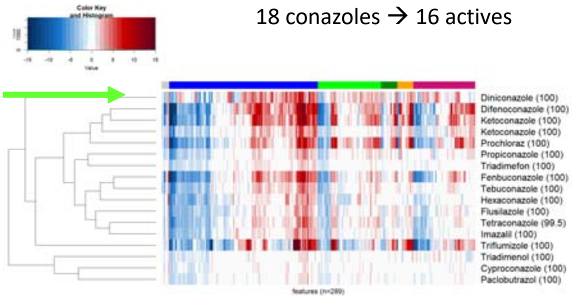
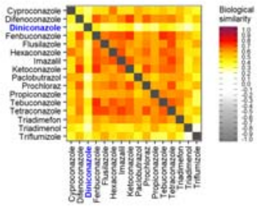
# Diniconazole – a peculiar conazole (I)

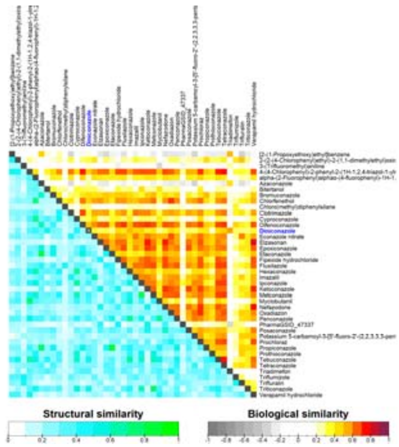
18 conazoles → 16 actives



re-test:

- new samples
- different vendors
- +41 other conazoles




⇨ **Diniconazole is phenotypically different from all other tested conazoles**

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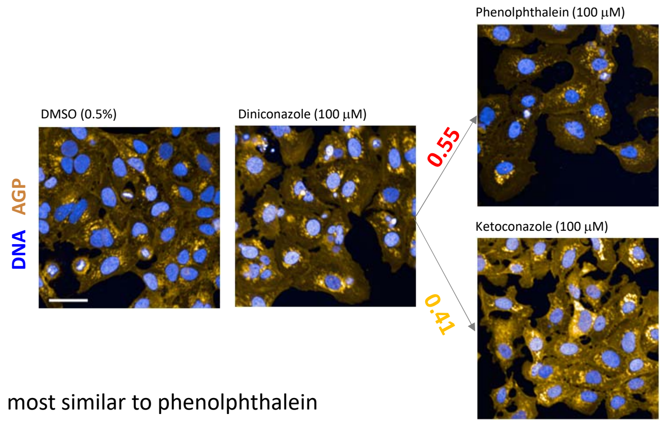
# Diniconazole – a peculiar conazole (II)

Chemicals phenotypically similar to diniconazole:

| | DTXSID | Chemical Name | Conc [uM] | Biol Sim |
|---|---|---|---|---|
| 1 | DTXSID0021125 | Phenolphthalein | 100 | 0.550 |
| 2 | DTXSID2032398 | Fludioxonil | 30.1 | 0.547 |
| 3 | DTXSID2032550 | Fenpyroximate (Z,E) | 100 | 0.546 |
| 4 | DTXSID8046666 | Methyl Violet | 3 | 0.535 |
| 5 | DTXSID2037712 | 4,4',4''-Ethane-1,1,1-triyltriphenol | 100 | 0.516 |
| 6 | DTXSID1024338 | Thiophanate-methyl | 100 | 0.511 |
| 7 | DTXSID4022442 | Bisphenol B | 100 | 0.509 |
| 8 | DTXSID5020576 | 17alpha-Ethinylestradiol | 100 | 0.503 |
| 9 | DTXSID6032192 | Tri-o-cresyl phosphate | 100 | 0.491 |
| 10 | DTXSID9020114 | Auramine hydrochloride | 10 | 0.482 |
| 11 | DTXSID2023224 | Loratadine | 100 | 0.480 |
| 12 | DTXSID6021248 | Rotenone | 1 | 0.478 |
| 13 | DTXSID3020465 | Diethylstilbestrol | 30 | 0.471 |
| 14 | DTXSID1021243 | Rhodamine 6G | 10 | 0.458 |
| 15 | DTXSID8029868 | Atorvastatin | 94.5 | 0.456 |
| 16 | DTXSID9032379 | Dithiopyr | 99.6 | 0.455 |
| 16 | DTXSID4020870 | 2,2'-Methylenebis(4-methyl-6-tert-butylphenol) | 30 | 0.455 |
| 16 | DTXSID1034181 | Raloxifene hydrochloride | 30 | 0.455 |
| 19 | DTXSID8041248 | 2,5-Di-tert-butylbenzene-1,4-diol | 100 | 0.451 |
| 20 | DTXSID0020573 | 17beta-Estradiol | 100 | 0.449 |
| 21 | DTXSID5032525 | Bifenazate | 100 | 0.448 |
| 21 | DTXSID7024081 | Endothal | 100 | 0.448 |
| 23 | DTXSID1026900 | Dodecyltrimethylammonium chloride | 30 | 0.443 |
| 24 | DTXSID1033664 | 17-Methyltestosterone | 100 | 0.436 |
| 25 | DTXSID9032113 | Tebuconazole | 100 | 0.435 |
| 26 | DTXSID6020226 | Cadmium chloride | 100 | 0.429 |
| 27 | DTXSID9032329 | Bensulide | 100 | 0.423 |
| 28 | DTXSID8032548 | Fenbuconazole | 100 | 0.422 |
| 29 | DTXSID5023871 | Amitraz | 100 | 0.420 |
| 30 | DTXSID7029879 | Ketoconazole | 100 | 0.417 |




- most similar to phenolphthalein
- many estrogenic chemicals are top ranked
  → is diniconazole estrogen-like?
- off-target effect of many estrogens is disruption of microtubules
  ⇨ hypothesis: diniconazole acts as a microtubule disruptor in U-2 OS cells

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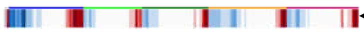
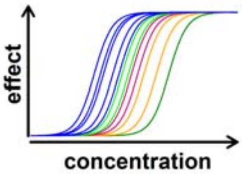
## Two Applications




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

**Application 1**

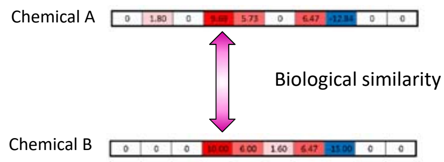
concentration-response modelling




⇨ **HTPP detects bioactive chemicals**
⇨ **use case: Bioactivity-Exposure Ratio**

Compare profiles with annotated reference chemicals → putative mechanisms

**Application 2**




⇨ **phenotypic profiles contain mechanistic information**
⇨ **structural similarity → phenotypical similarity
But HTPP can identify important exceptions**
⇨ **Prediction of new/unkown mode-of-action**



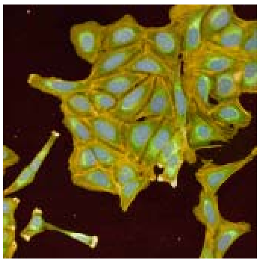
## Outlook: US EPA

- Application of HTPP in multiple human cell lines
- Comparison to transcriptomics data

## Outlook: Jo's group at UFZ

- Application of HTPP to non-human species

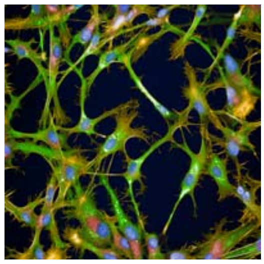
human-centric



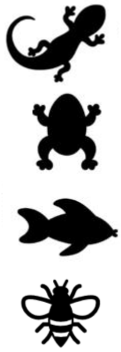
Human osteosarcoma cells
Adapted from Nyffeler *et al.* 2020

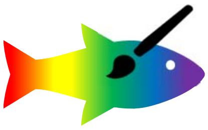
- image-based methods
- high-throughput

ecologically
relevant species



Rainbow trout gill cells
Adapted from Nyffeler *et al.* 2022





**# tested chemicals ↗**
**# represented species ↗**

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## Acknowledgements

**Office of Research and Development (ORD)**
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Harrill Lab team
- **Joshua Harrill**
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- Richard Judson
- Ryan Lougee
- Grace Patlewicz
- Katie Paul Friedman
- Ann Richard
- Woody Setzer
- Imran Shah
- John Wambaugh

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- Scott Auerbach

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# Thank you for your attention!