EPA
United States
Environmental Protection
Agency

Phenotypic Profiling for High-Throughput Chemical Bioactivity Screening at the U.S. EPA

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Disclaimer

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

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Overview

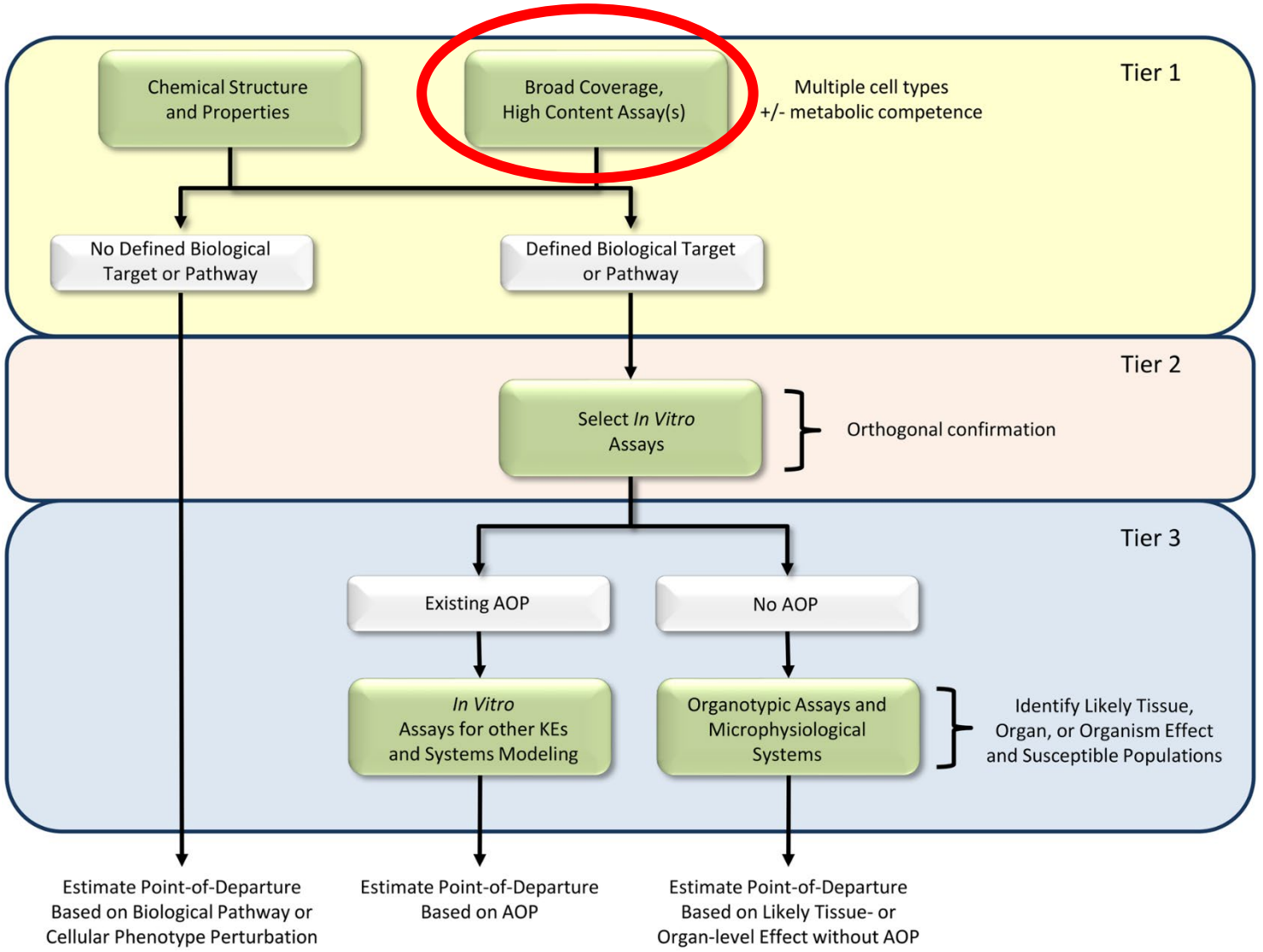
1. EPA's Tiered testing framework for hazard characterization
2. What is (phenotypic) profiling?
3. Application 1: Potency estimates
4. Application 2: Mechanistic information

Tiered testing framework for hazard characterization

The Next Generation Blueprint of Computational Toxicology at the U.S. Environmental Protection Agency
Thomas et al. 2019
Toxicological Sciences, Volume 169, Issue 2, June 2019, Pages 317–332

Two profiling assays:

- transcriptomics
- phenotypic profiling



EPA United States Environmental Protection Agency

What is (phenotypic) profiling?

What does ‘profiling’ mean?

Targeted assays

Example: Estrogen receptor agonist assay (NVS_NR_hER)

- Response: decreased radioligand binding
- Positive control: 17b-estradiol
- Number of endpoints: 1

→ For active chemicals, the response is a predictable change in a single endpoint in a known direction

Profiling assays

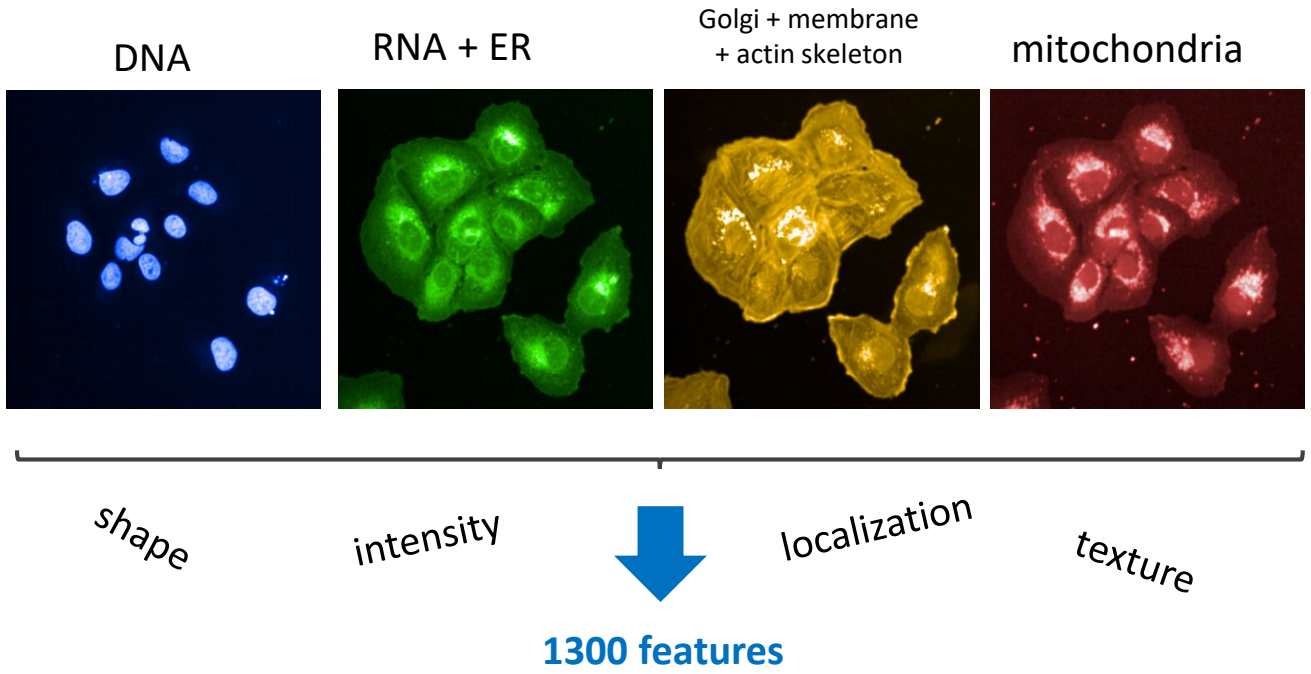
Example: Transcriptomics

- Response: any meaningful change in transcript levels
- Number of ‘endpoints’: ~ 10,000

→For active chemicals, responses involve changes in many different endpoints in unknown directions. Vary from chemical-to-chemical.

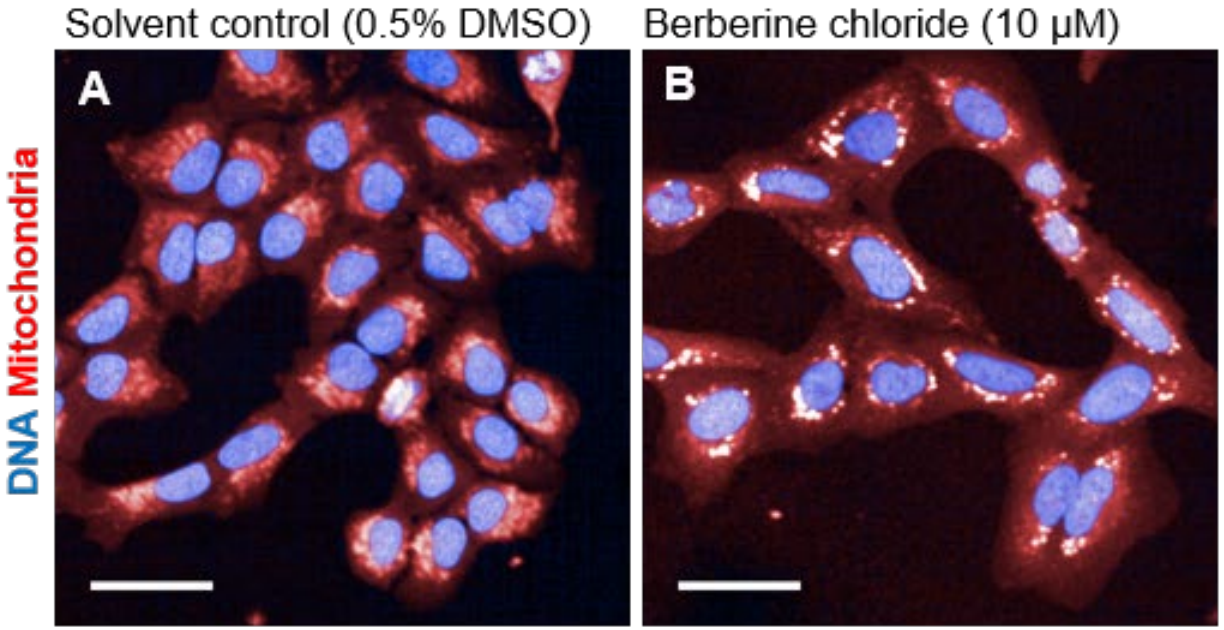
What is imaging-based phenotypic profiling?

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

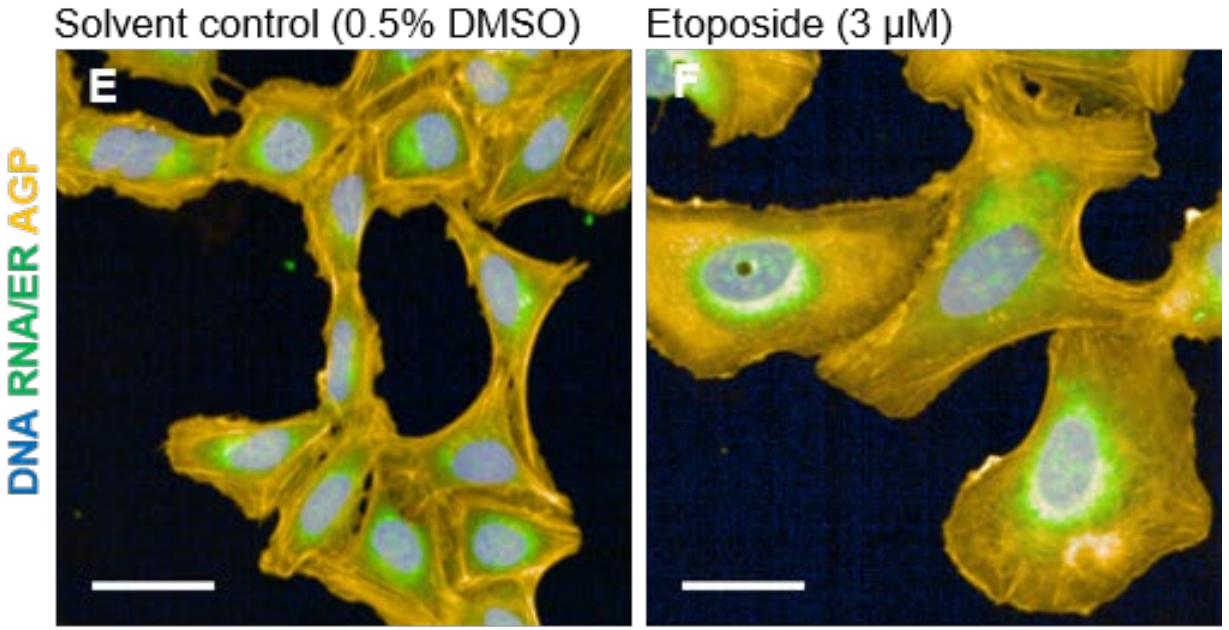


Cell Painting = Cytological Profiling = Phenotypic Profiling = high-throughput Phenotypic Profiling = HTPP

Exemplary chemicals



→ Mitochondrial compactness/texture

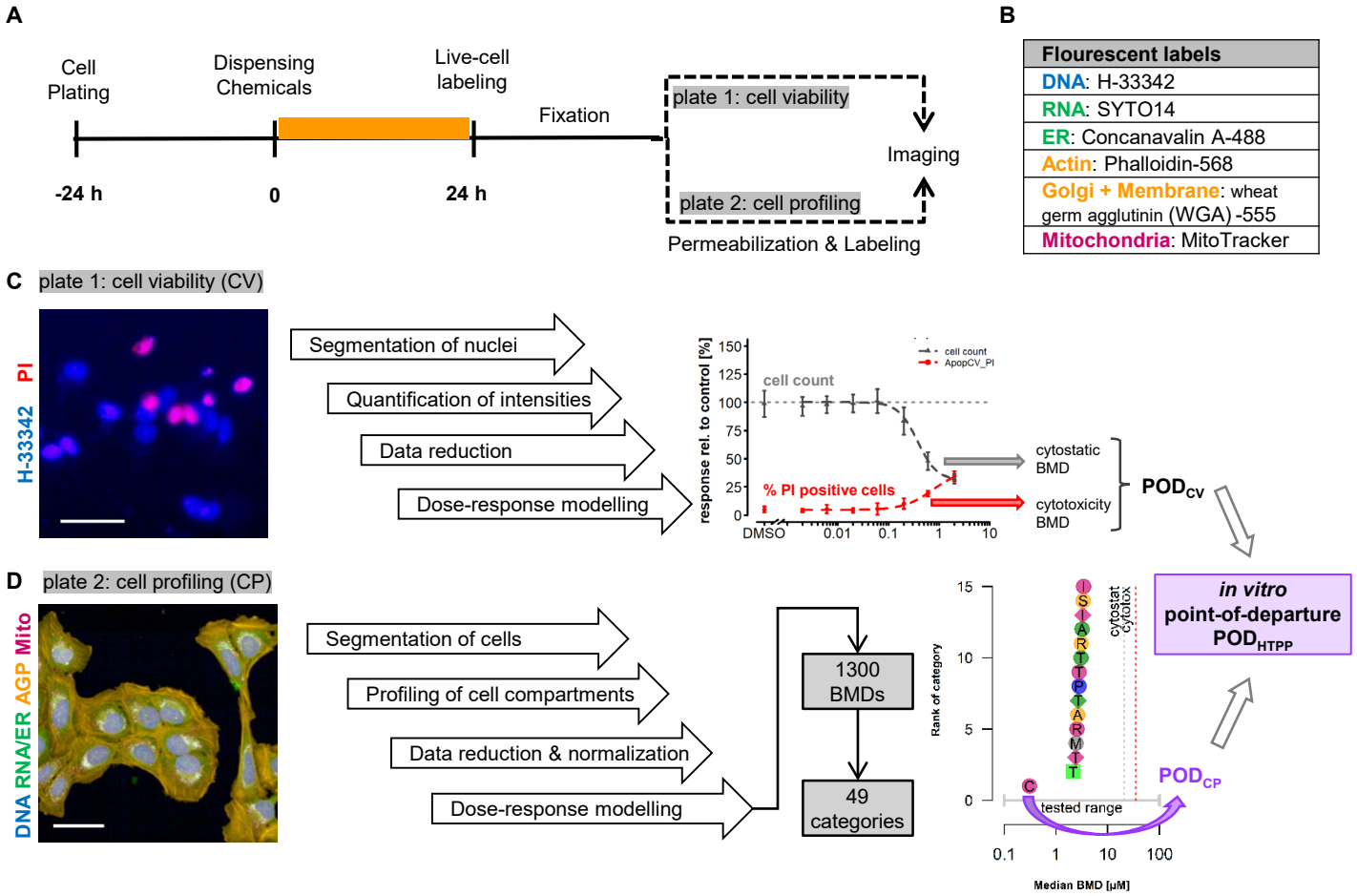


→ Cells are larger

⇨ **Strong phenotypes are observable qualitatively**

adapted from Nyffeler et al. 2020a

The High-Throughput Phenotypic Profiling (HTPP) assay



POD: point-of-departure
=
PAC: phenotype altering concentration

adapted from Nyffeler et al. 2020a

Image analysis workflow: image segmentation

1. find nuclei

2. find cell outline

3. reject border objects

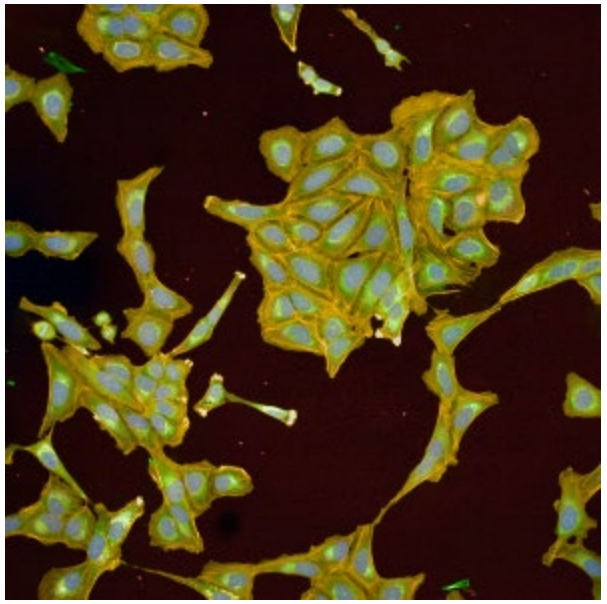
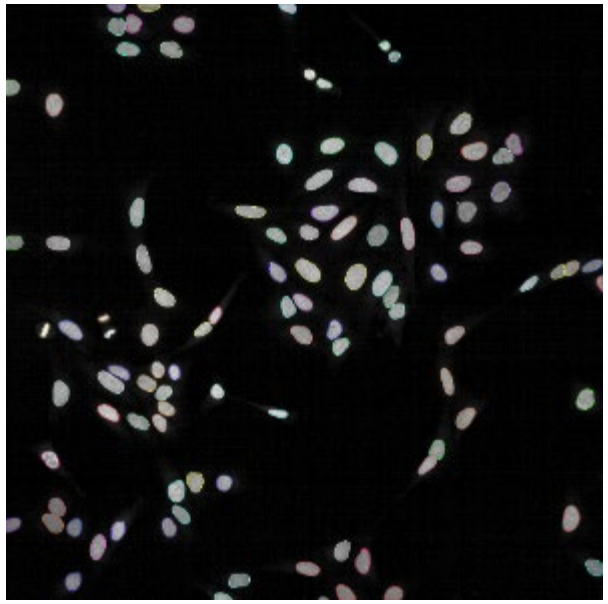
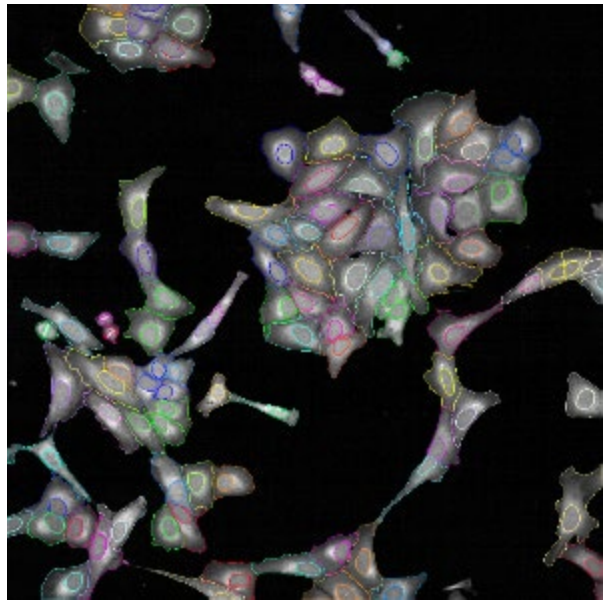
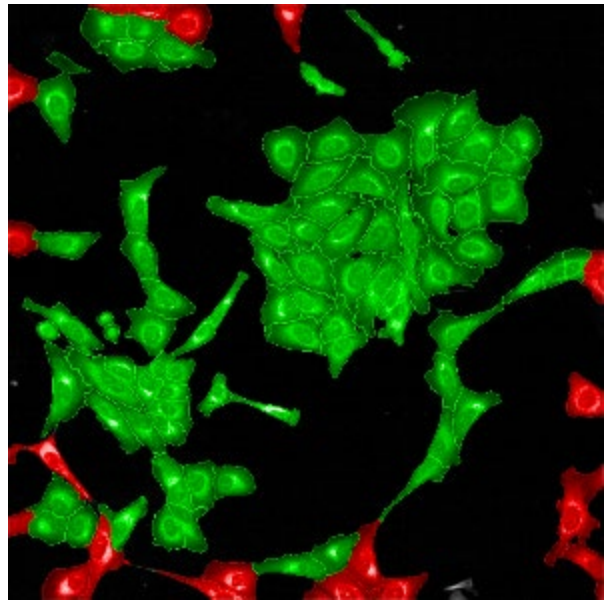
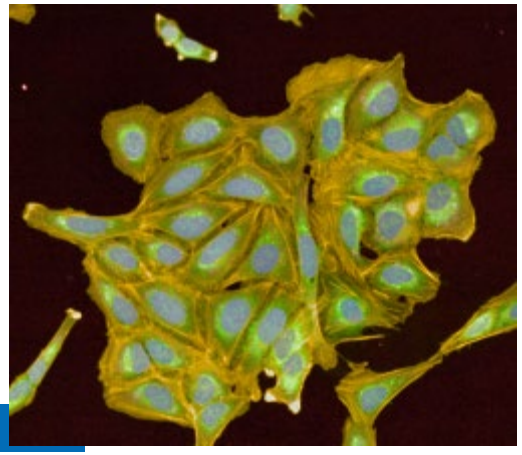
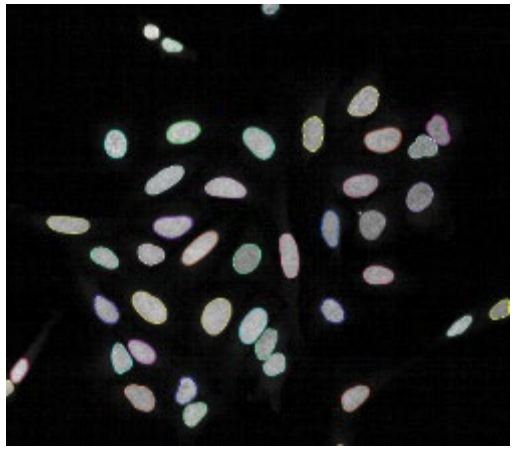
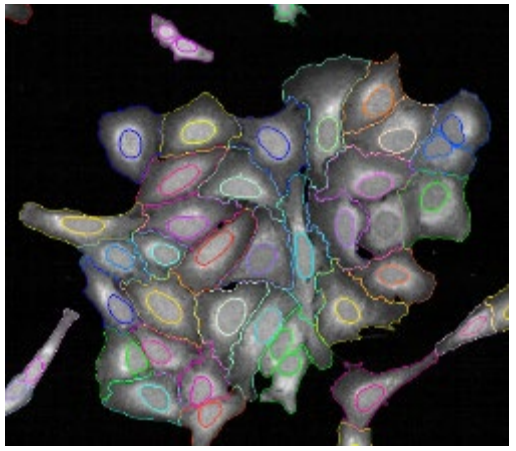
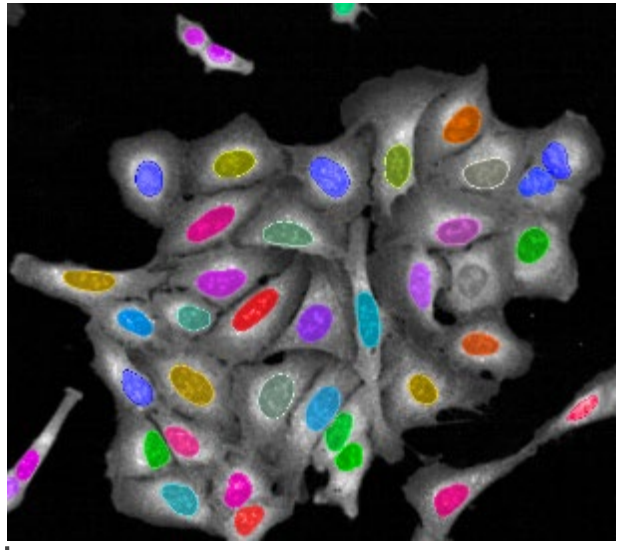
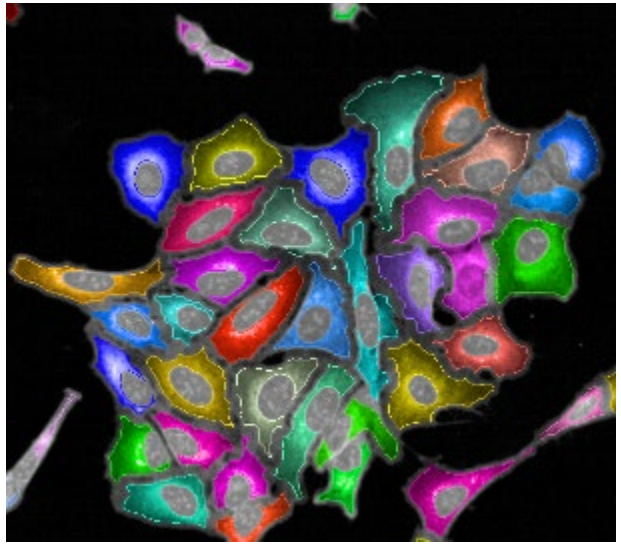








Image analysis workflow: define cellular compartments

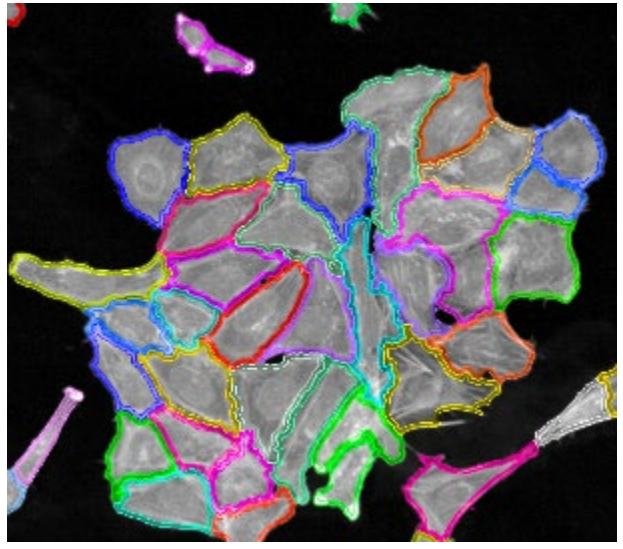
nuclei



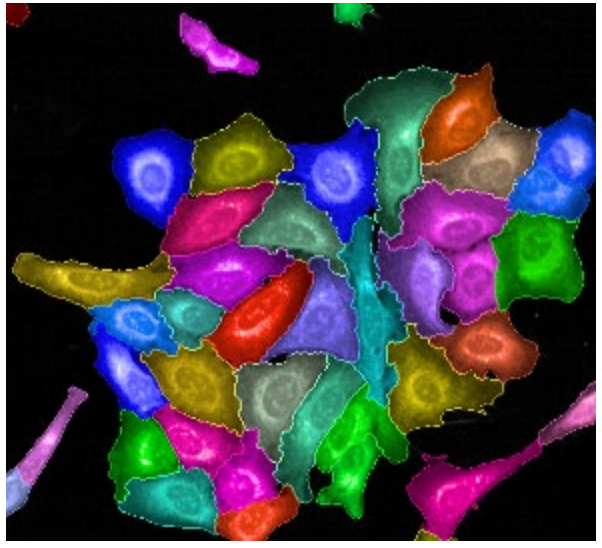
cytoplasm



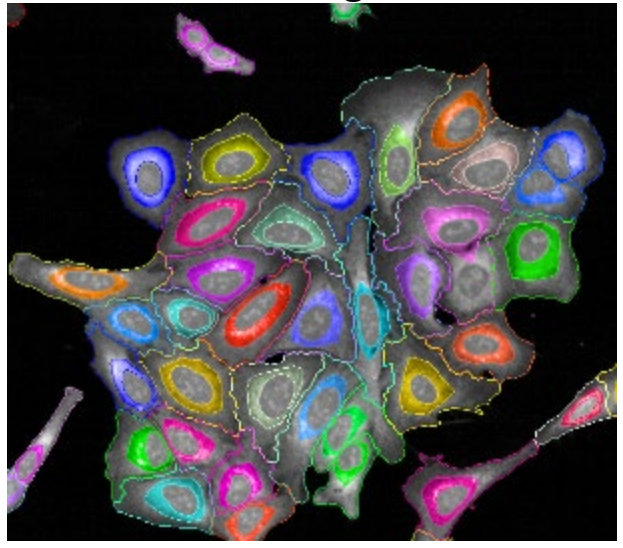
membrane



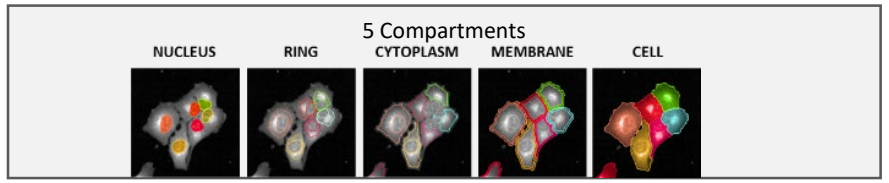
cell

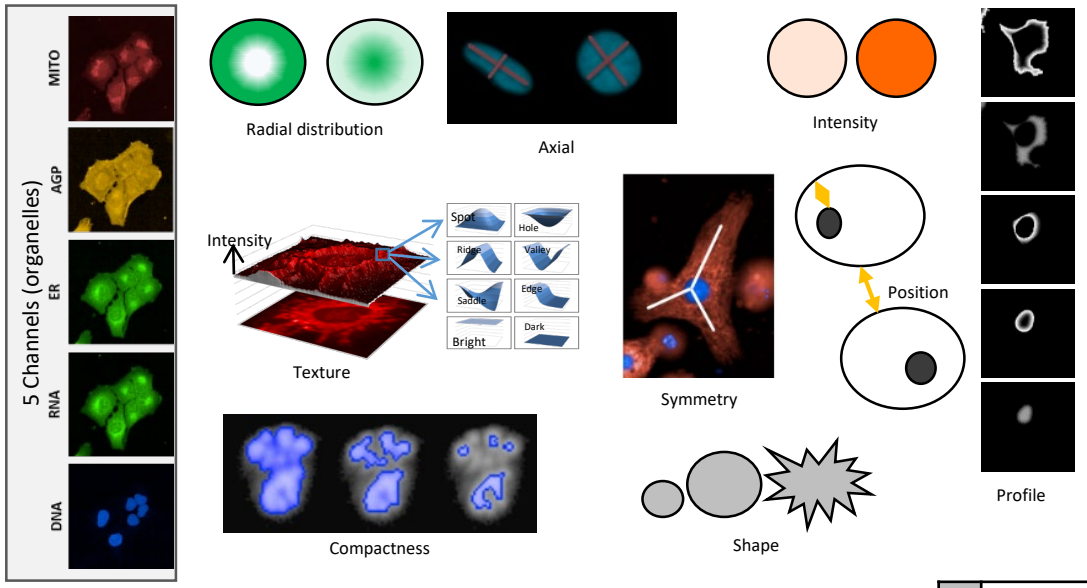


ring



Phenotypic feature extraction





49 feature categories
(ex. Mito_Texture_Cytoplasm)

1300 features / cell

		Module								
		Position [7]	Basic morph-ology [5]	SCARP morphology					Intensity [9]	Texture [14]
				Symmetry [80]	Compactness [40]	Axial [20]	Radial [28]	Profile [20-30]		
Channel	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							

PerkinElmer Opera Phenix

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

With illustrations from Perkin Elmer

Quality control of the CP assay

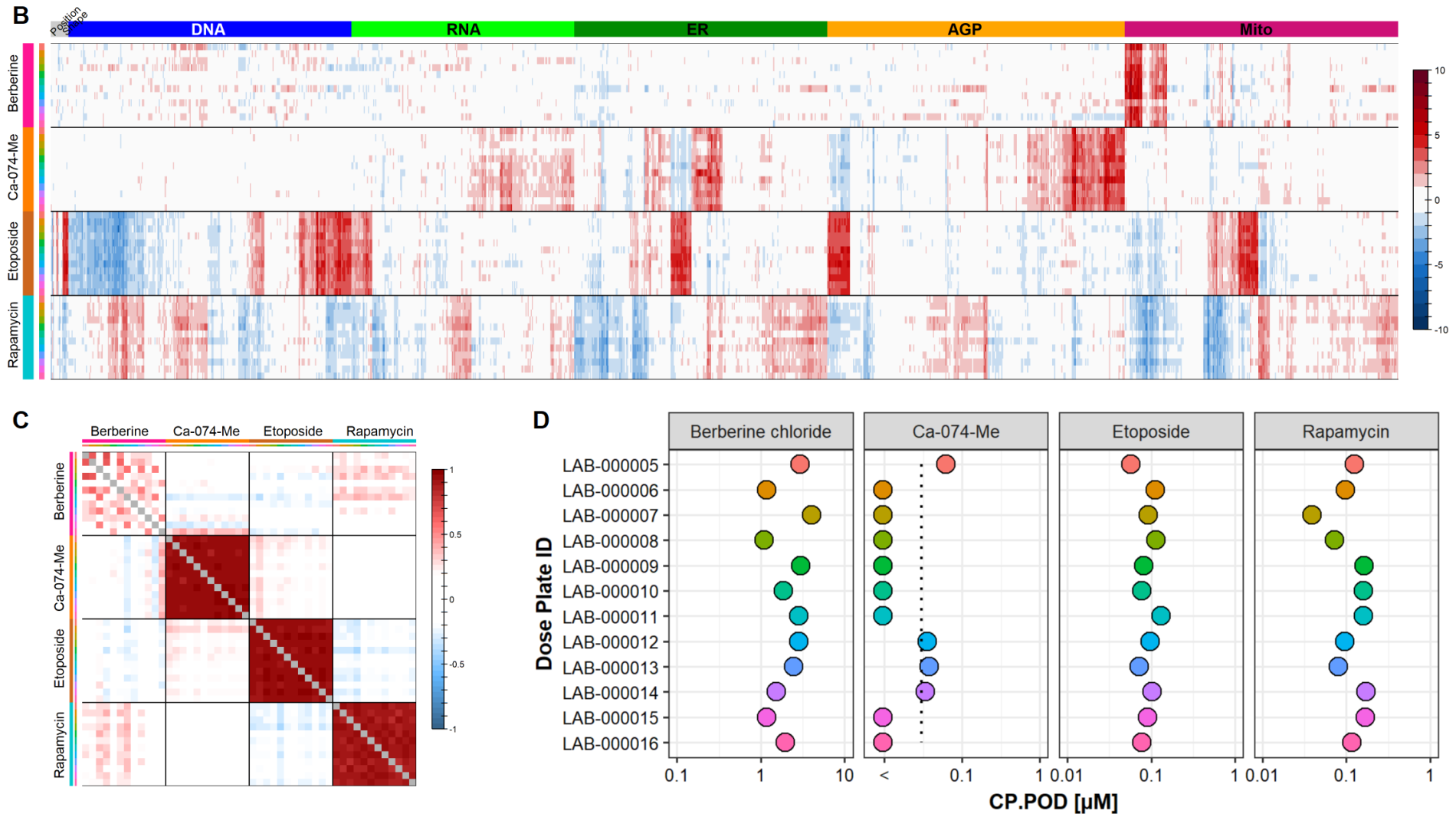


Figure 5

- ⇨ **Reproducible profile**
- ⇨ **PODs vary by less than 1 order of magnitude**

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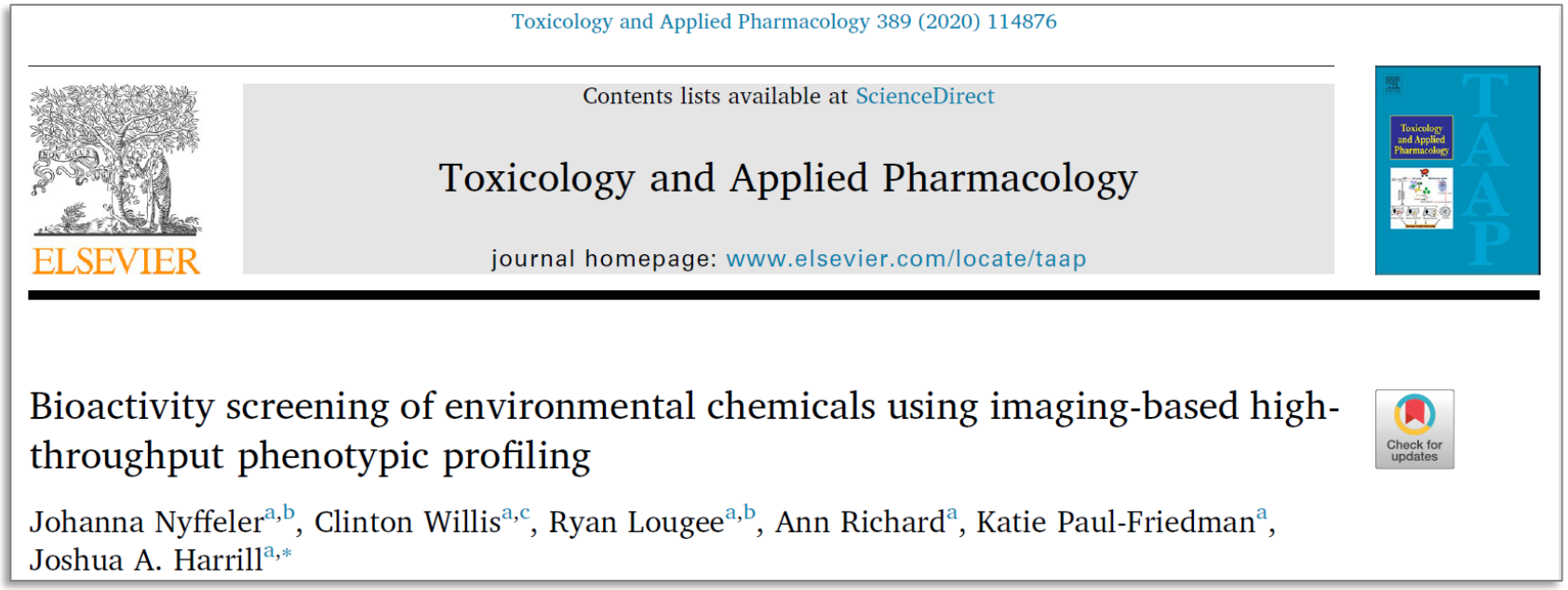
Application 1: Potency estimation



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Bioactivity screening of environmental chemicals using imaging-based high-throughput phenotypic profiling

Johanna Nyffeler[a,b], Clinton Willis[a,c], Ryan Lougee[a,b], Ann Richard[a], Katie Paul-Friedman[a], Joshua A. Harrill[a,*]

Screen of environmental chemicals

- 462 test chemicals
  - pesticides (~ 75%), drug-like chemicals, food additives, industrial chemicals
  - 448 chemical from the 'APCRA' list
    - available *in vivo* effect values
    - available toxicokinetic parameters for in vitro to in vivo extrapolation (IVIVE)

Experimental design	
Cell type	U-2 OS
Exposure time	24 h
Cell seeding density per well	400
# unique chemicals	**462**
# concentrations	8
Concentration spacing	½ $\log_{10}$
# solvent controls/plate	24
# replicates/plate	1
# independent experiments	4

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

How to analyze high-dimensional data?

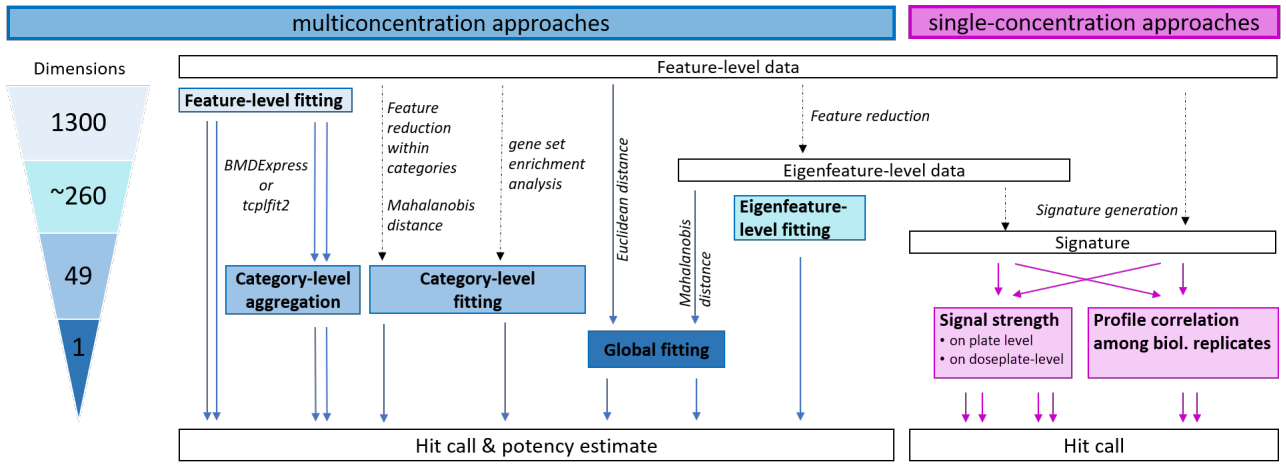
Original Research

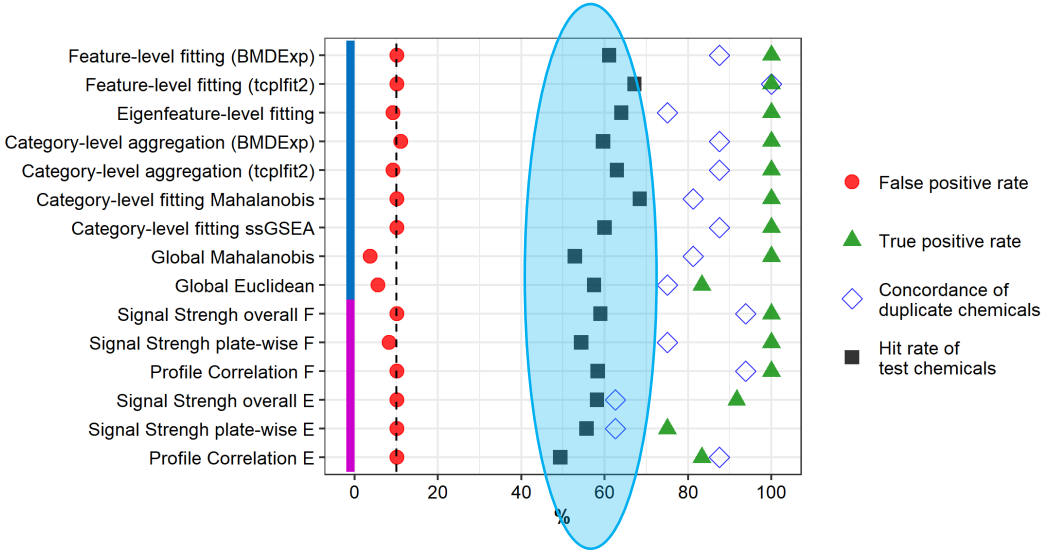
SLAS Discovery
1–17
© Society for Laboratory Automation and Screening 2020
DOI: 10.1177/2472555220950245
journals.sagepub.com/home/jbx
SAGE

Comparison of Approaches for Determining Bioactivity Hits from High-Dimensional Profiling Data

Johanna Nyffeler[1,2], Derik E. Haggard[1,2], Clinton Willis[1,3], R. Woodrow Setzer[1], Richard Judson[1], Katie Paul-Friedman[1], Logan J. Everett[1], and Joshua A. Harrill[1]

Analyzed it with 15 different analysis approaches

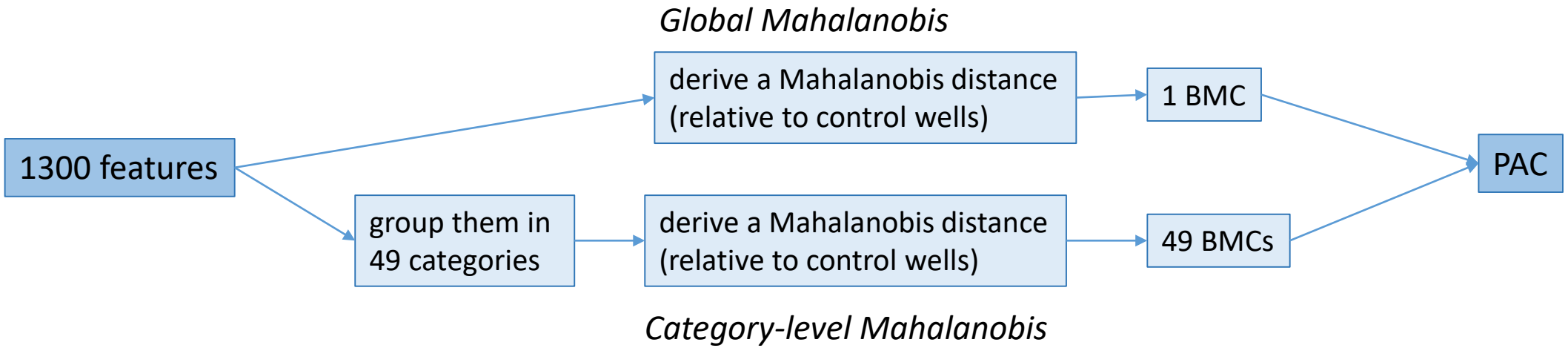




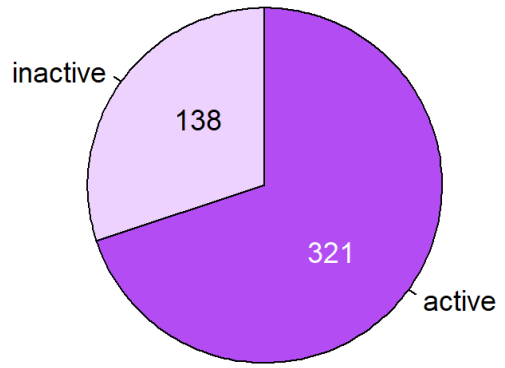
⇨ **With all approaches, 50-70% of the chemicals were identified as active**

How to analyze high-dimensional data?

- Two approaches were sensitive and reproducible:

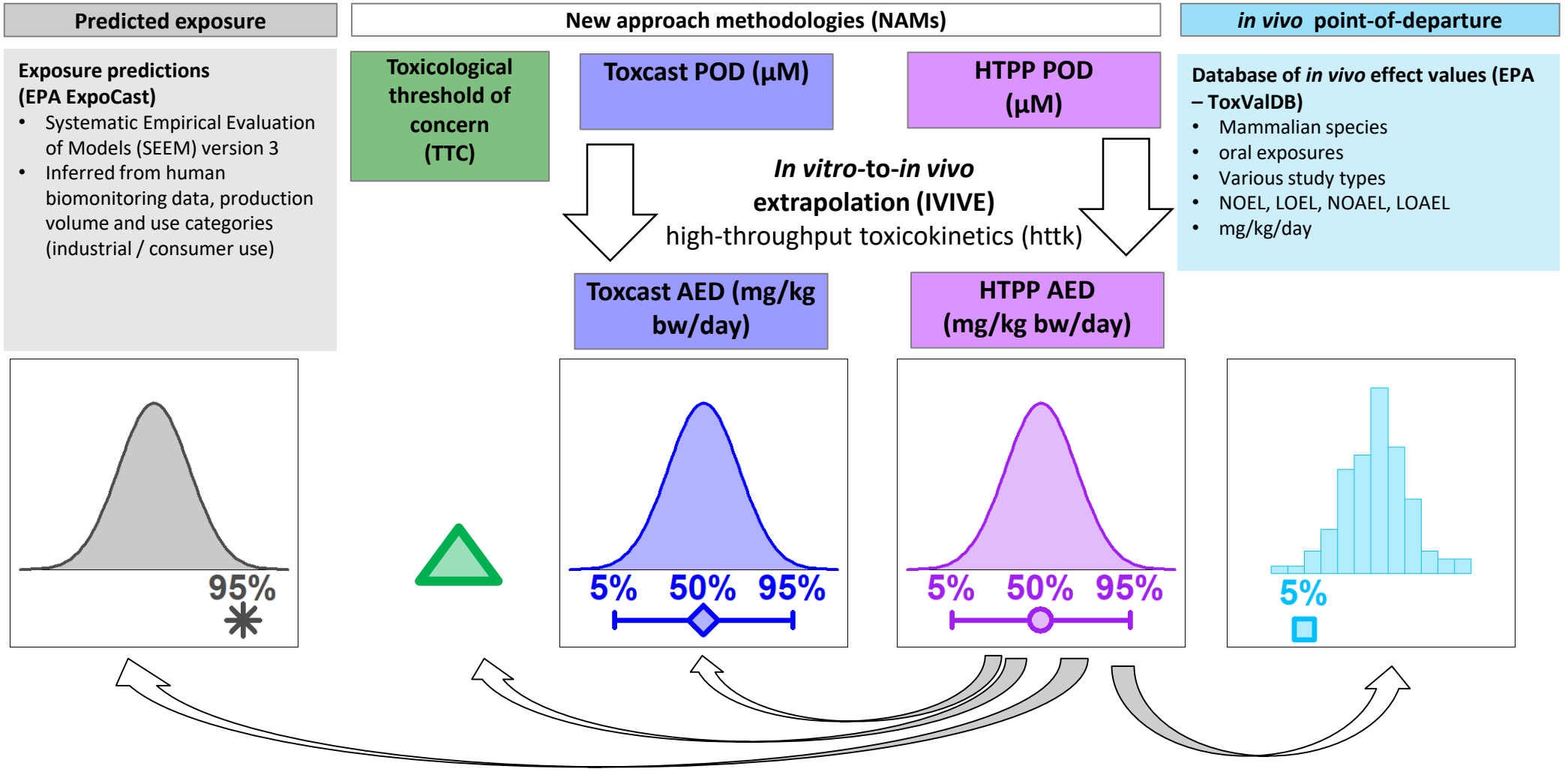


- Active = active in either one of the two approaches



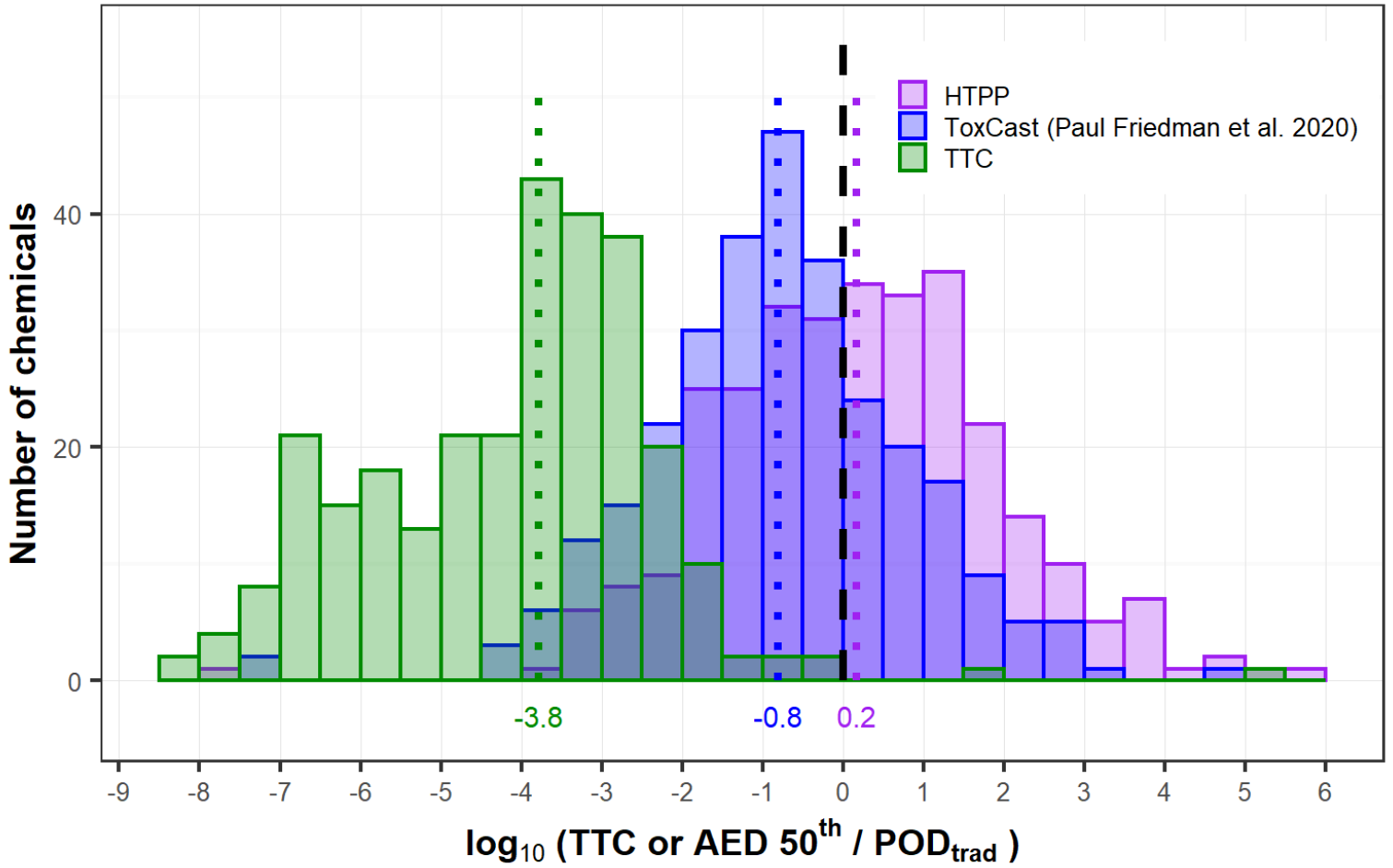
⇨ **70% of chemicals were active**

Comparison to *in vivo* data and exposure



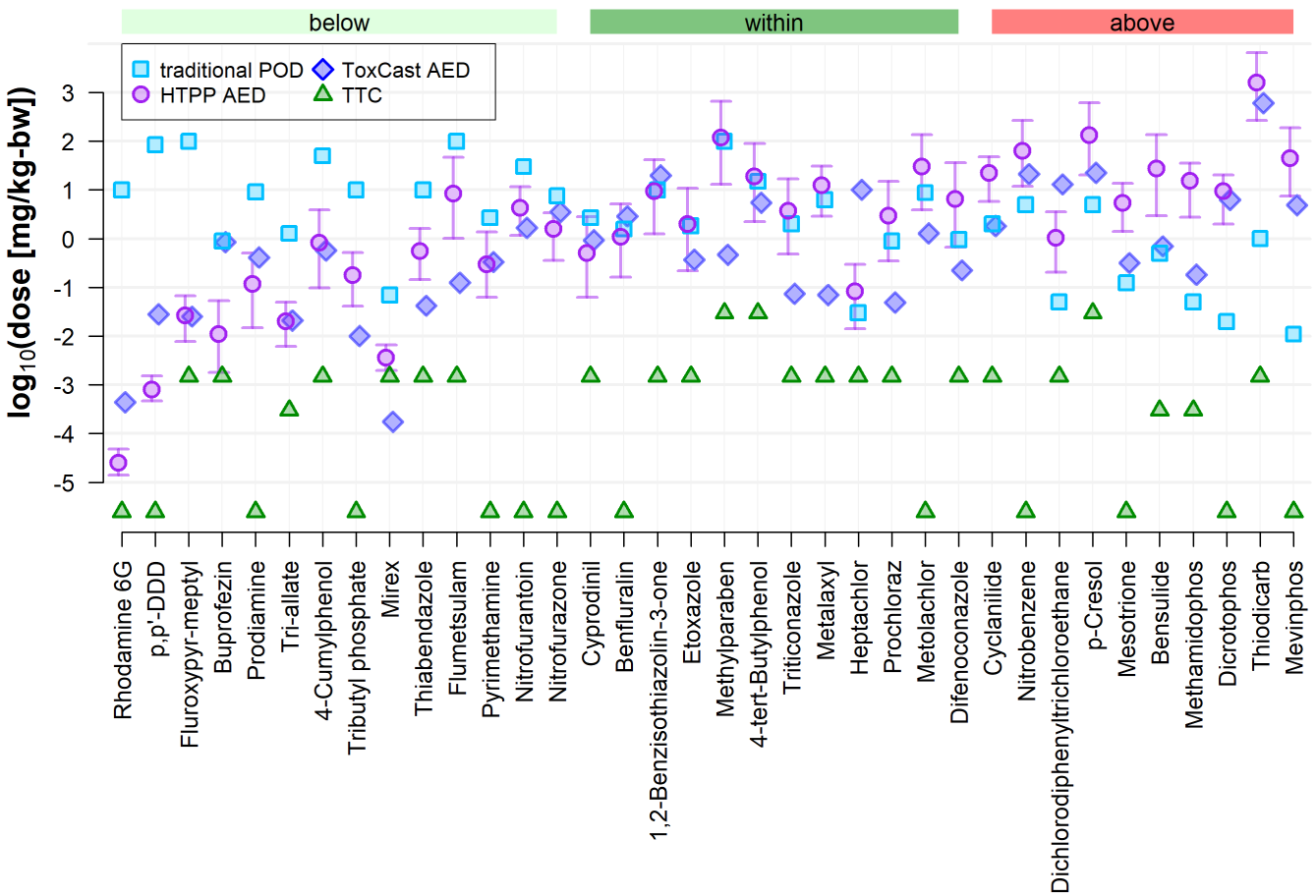
POD: point-of-departure
AED: administered equivalent dose

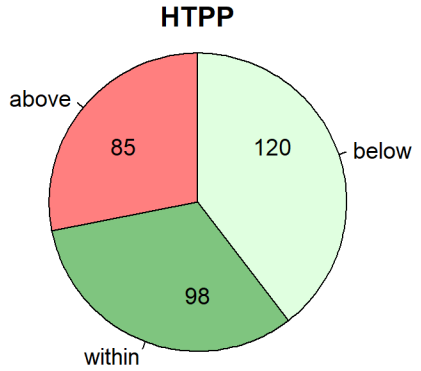
Comparison to *in vivo* effect values & other NAMs (I)

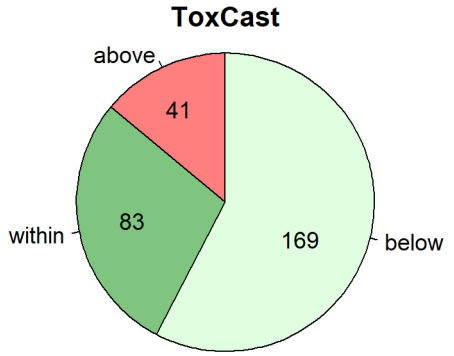


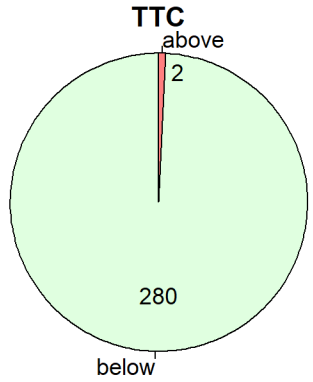
- ⇨ **HTPP AEDs are less potent than ToxCast-derived AEDs and TTC values**
- ⇨ **78% of HTPP AED are within 2 orders of magnitude of the *in vivo* POD**

Comparison to *in vivo* effect values & other NAMs (II)







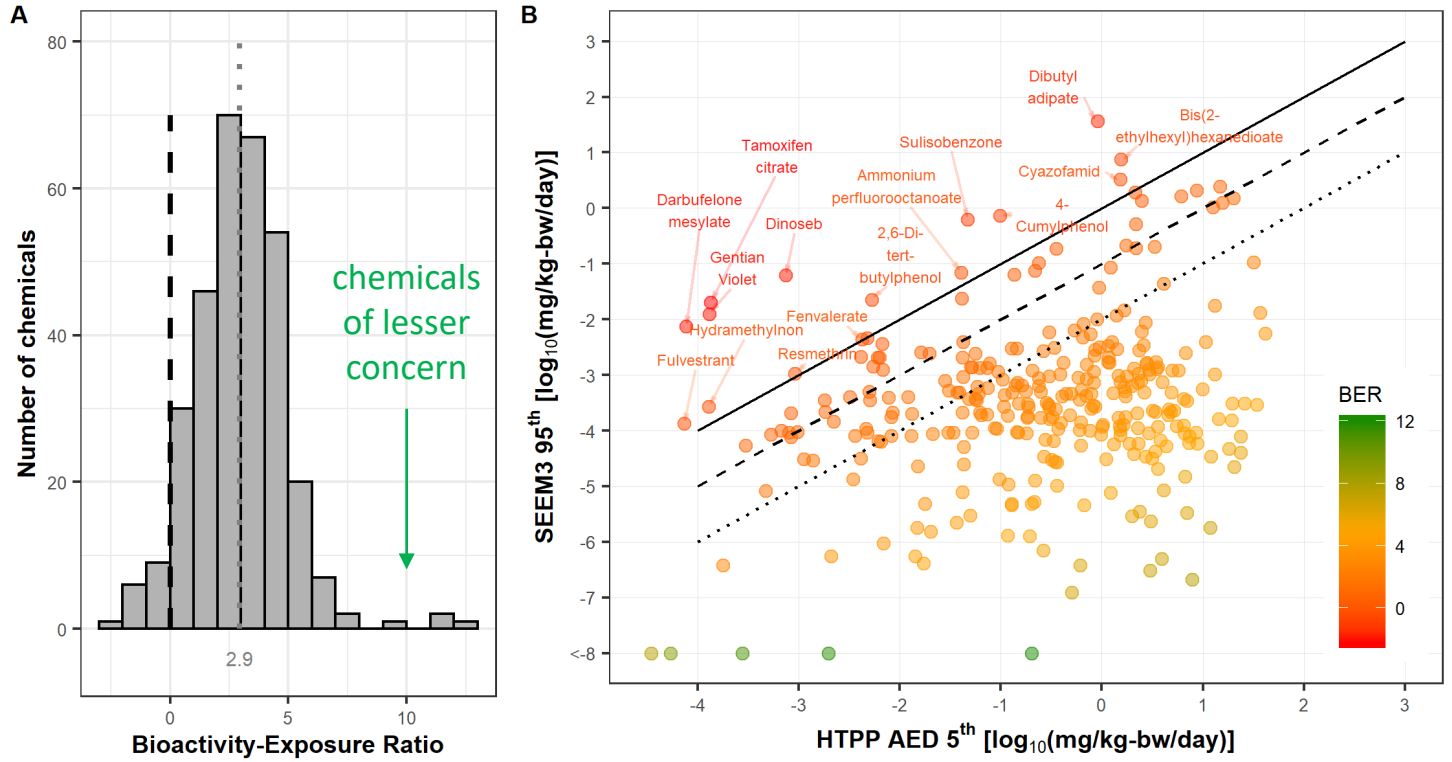


⇨ **for 72% (218/303) of chemicals, HTPP AEDs led to a conservative or comparable surrogate**

Comparison to exposure estimates

HTPP AEDs were compared to exposure predictions and the bioactivity exposure ratio was calculated as follows:

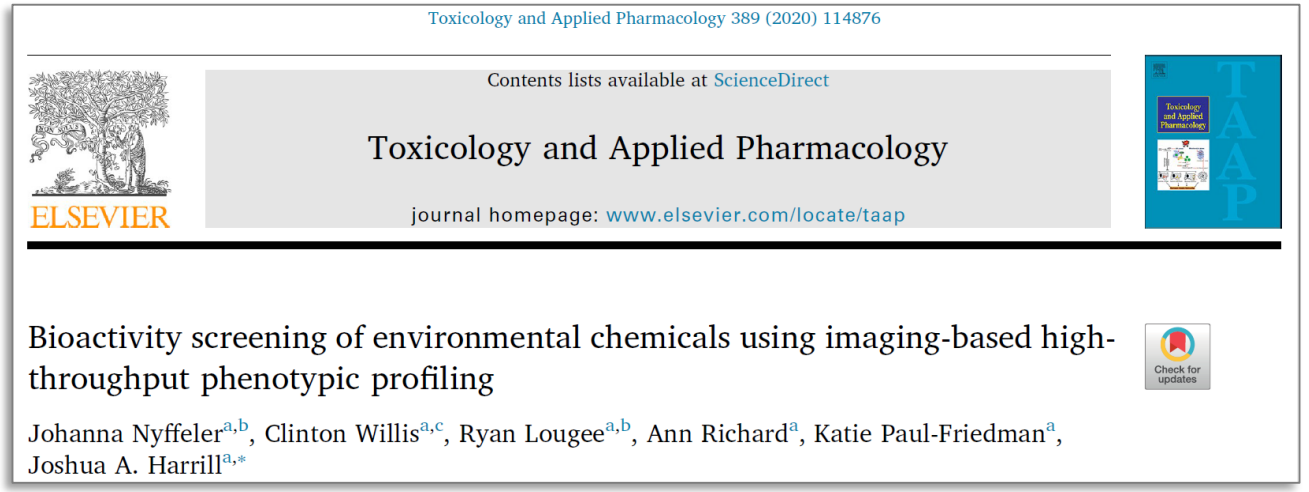
$$\text{Bioactivity exposure ratio (BER)} = \frac{\text{lower bound of HTPP bioactivity}}{\text{upper bound of exposure estimate}} = \log_{10}\left(\frac{\text{HTPP AED } 5^{\text{th}}}{\text{SEEM3 } 95^{\text{th}}}\right)$$



unpublished

- ⇨ **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**

Conclusions I



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HTPP *in vitro* potencies can be used for prioritizing of chemicals based on inferred bioactivity in relation to predicted human exposure

Next steps:

- Test chemicals in multiple cell types to increase biological coverage

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Application 2: Identification of putative mode-of-actions

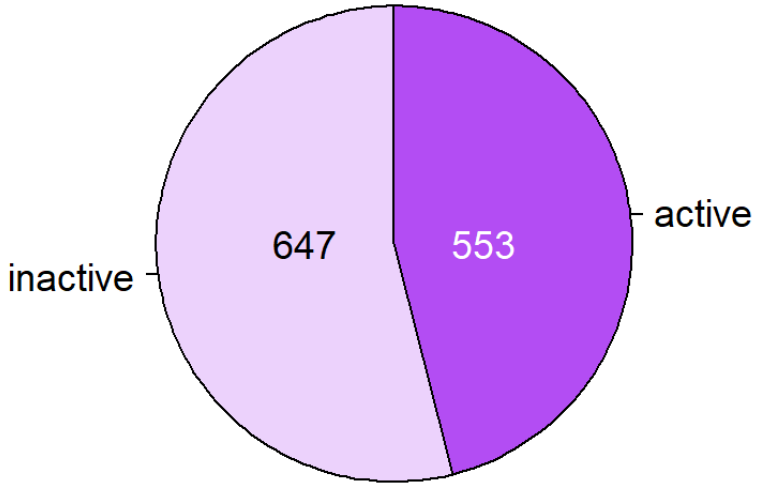
work in progress

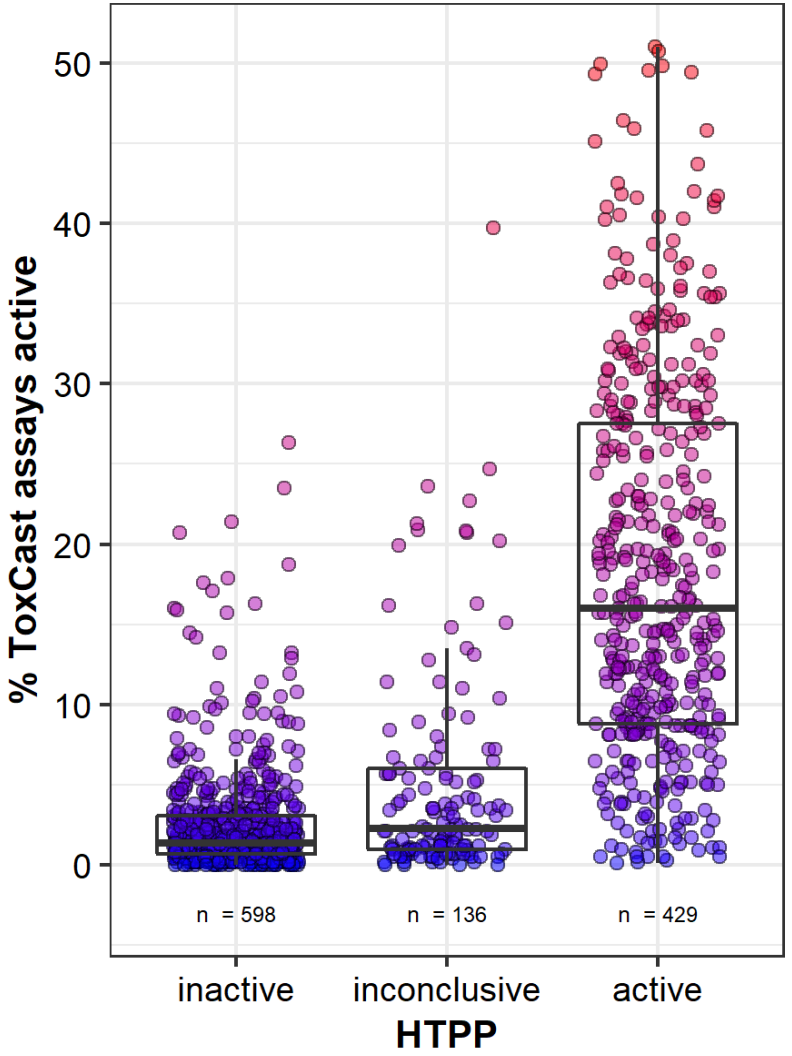
Screen of environmental & ToxCast chemicals

- 1201 chemicals
  - 442 were also in the previous screen, inclusive of APCRA chemicals
  - 179 were annotated with a target in RefChemDB (Judson et al. 2019)
  - Many chemicals in the set are of interest to the Agency under TSCA

Experimental design	
Cell type	U-2 OS
Exposure time	24 h
Cell seeding density per well	3000
# unique chemicals	**1201**
# concentrations	8
Concentration spacing	½ $\log_{10}$
# solvent controls/plate	18
# replicates/plate	1
# independent experiments	4

Screening results (I)

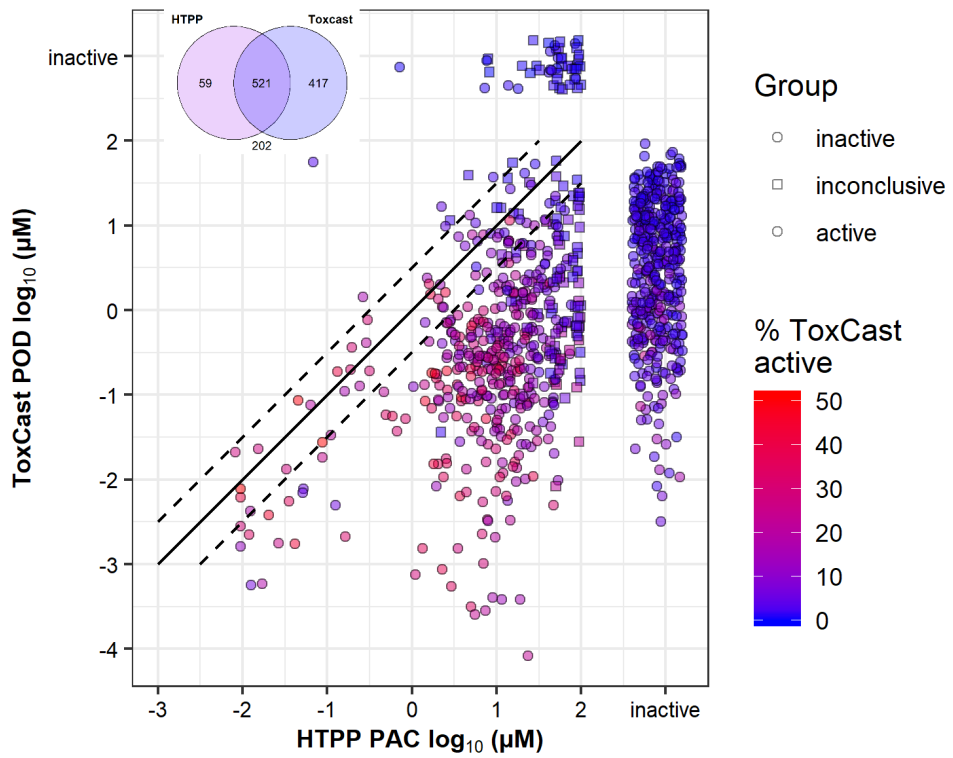




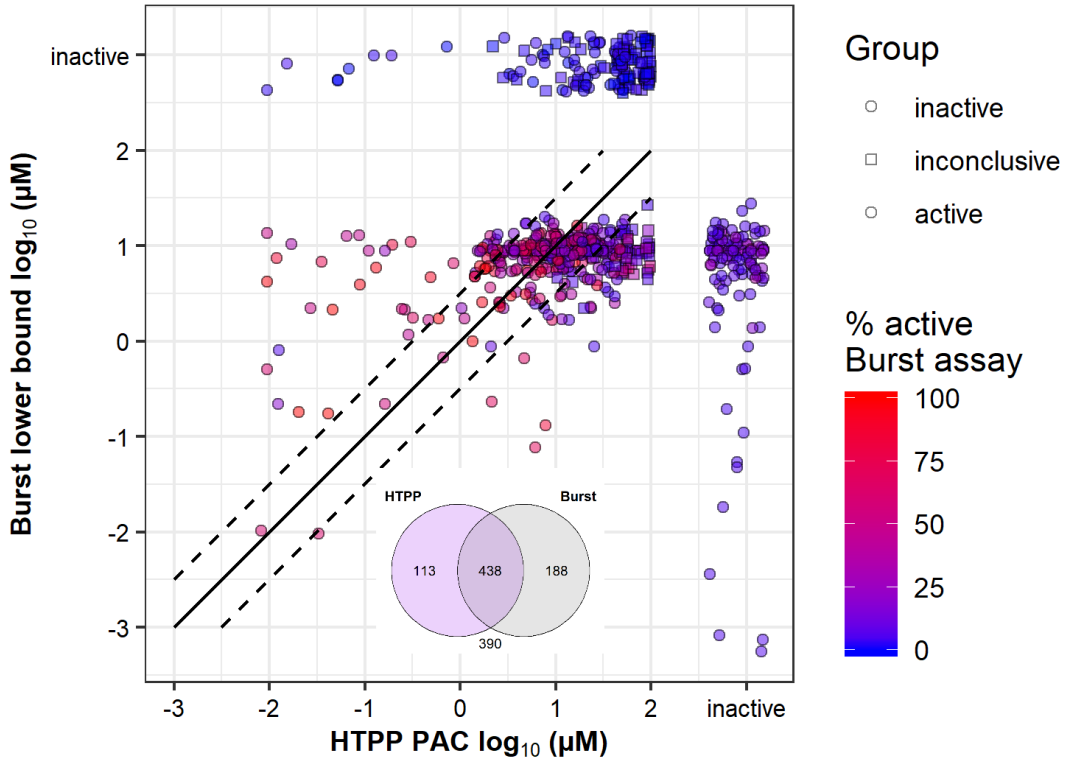
⇨ **Chemicals active in HTPP are more often ‘promiscuous’ in ToxCast**

Screening results (II)

Comparison with ToxCast screening results:

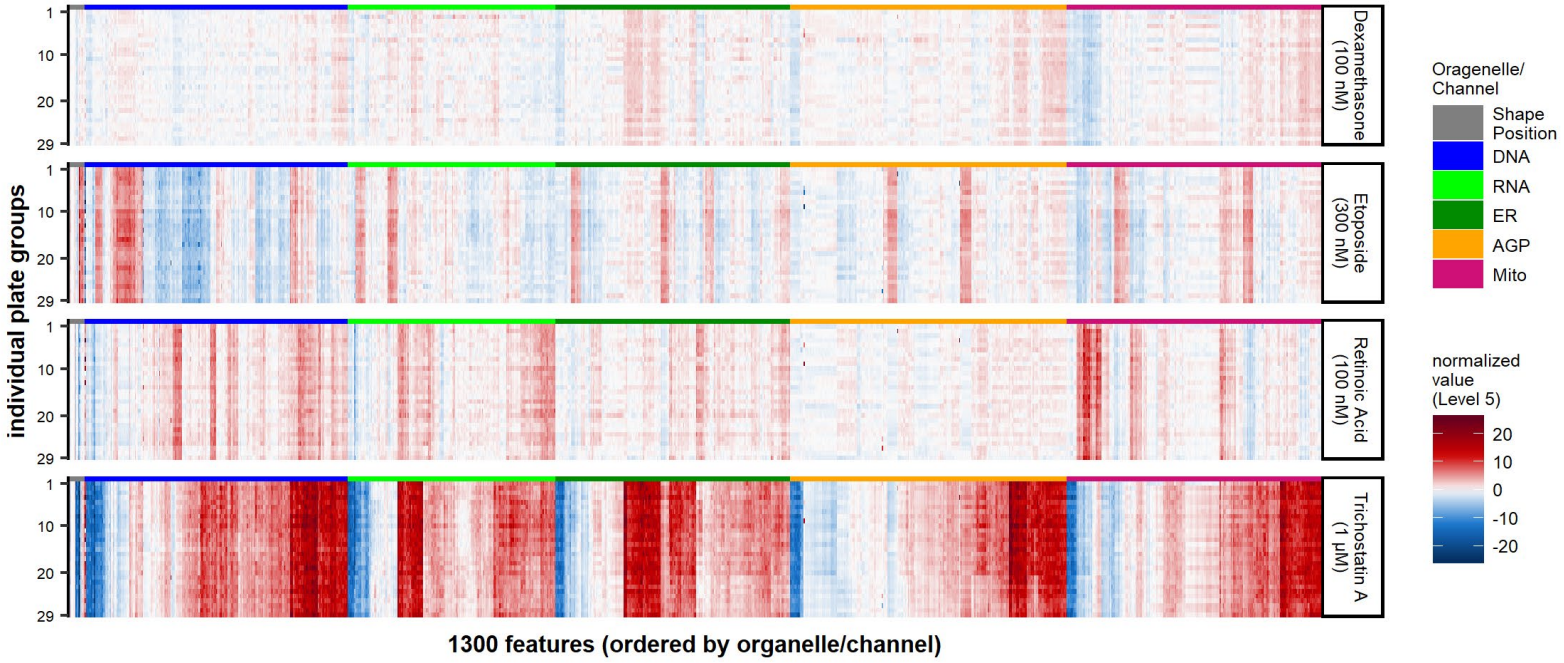


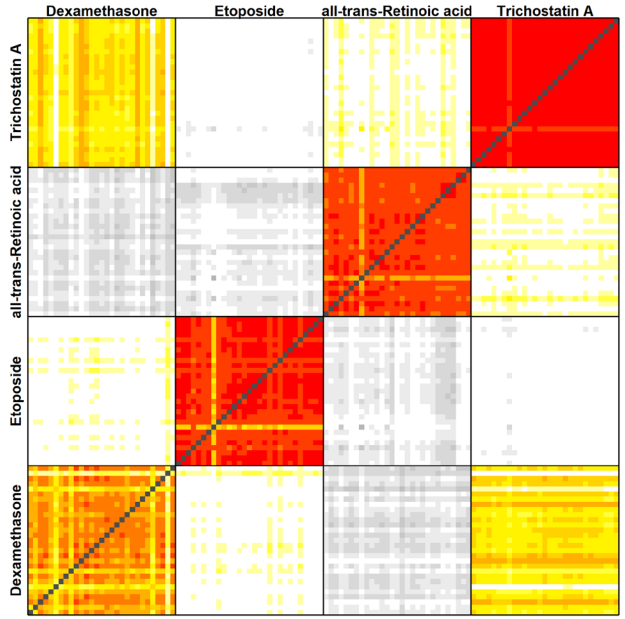
⇨ **less potent than ToxCast POD**



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

Compare phenotypic profiles





⇨ **Reference chemicals produce <u>reproducible</u> and <u>distinct</u> profiles.**

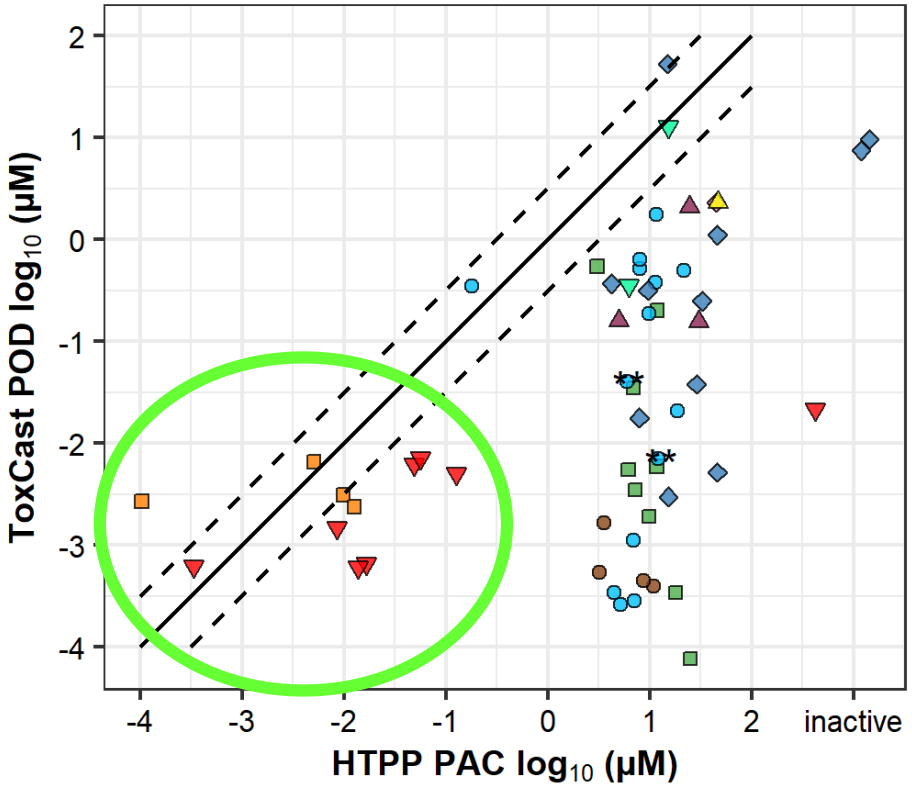
Example: Nuclear Receptor Modulators (I)

- 52 chemicals were annotated as targeting a nuclear receptor

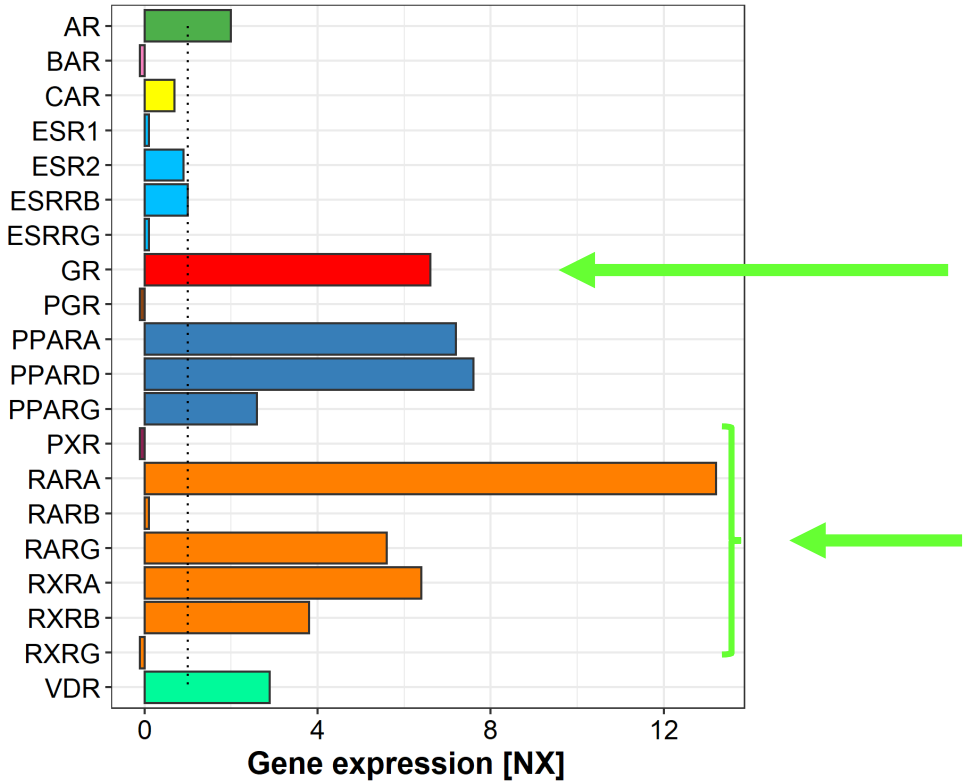
target

AR
BAR
CAR
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**

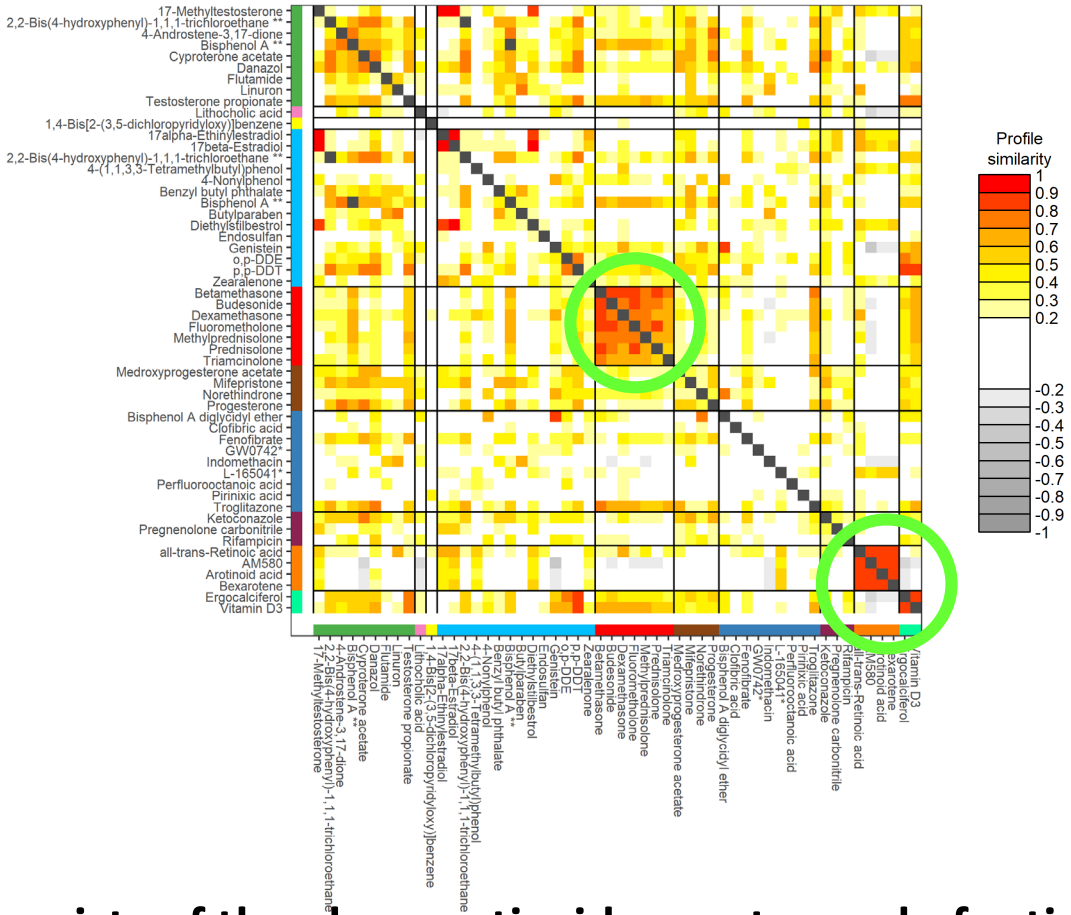
⇨ **For all other receptors, we are much less sensitive than ToxCast (off-target effects?)**

Example: Nuclear Receptor Modulators (II)

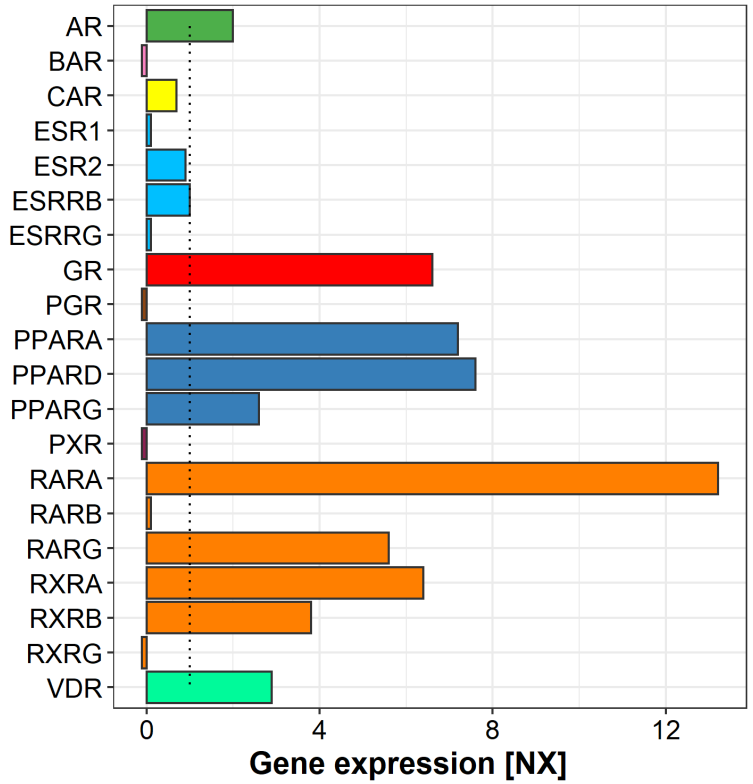
target

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

Biological similarity in HTPP



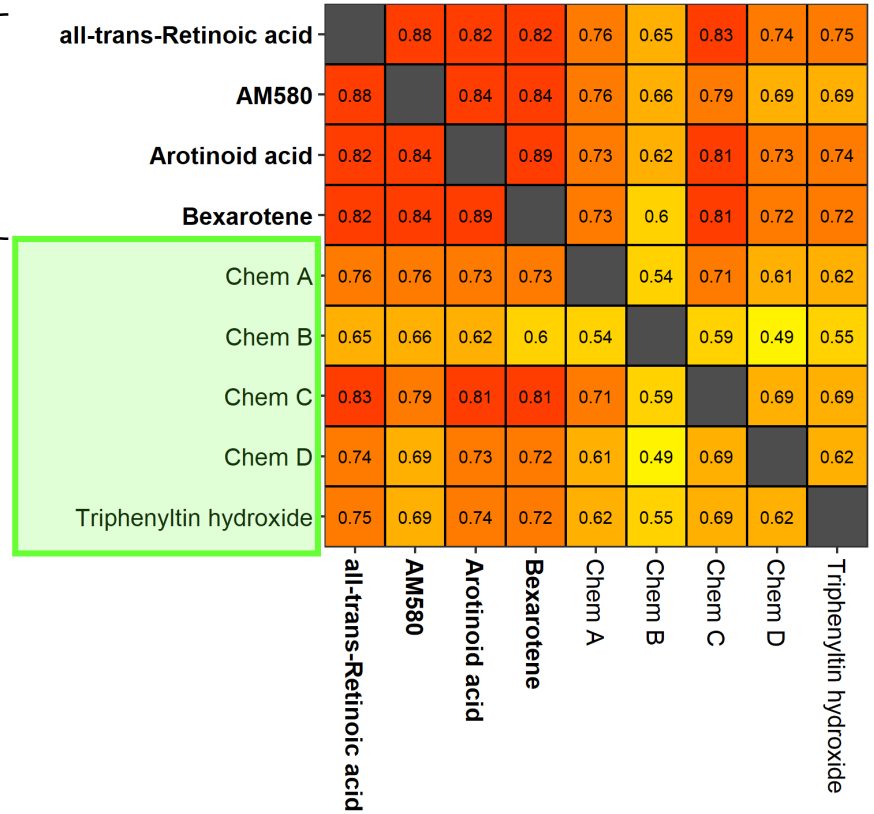
Gene expression in U-2 OS

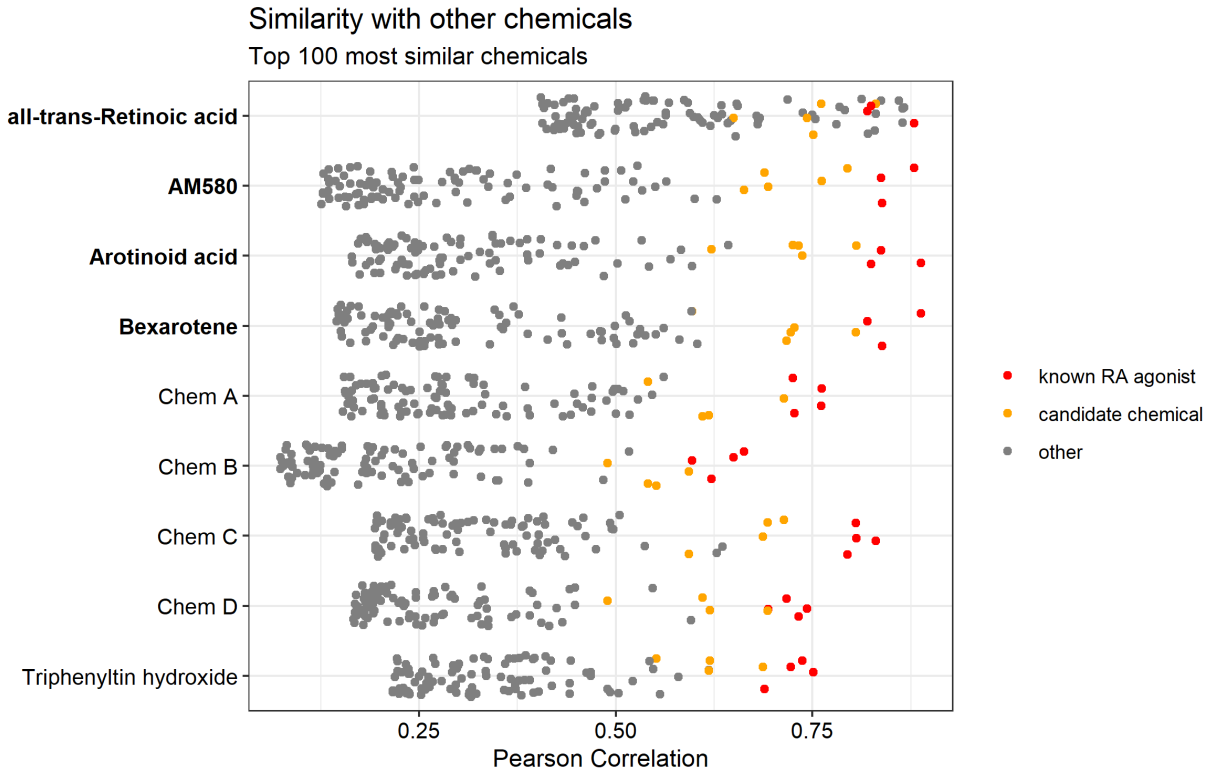


- Agonists of the glucocorticoid receptor and of retinoic acid receptors display characteristic profiles
- Expression of a target does not guarantee that characteristic profiles are observed (e.g. PPAR)

Identify test chemicals with similar profiles (I)

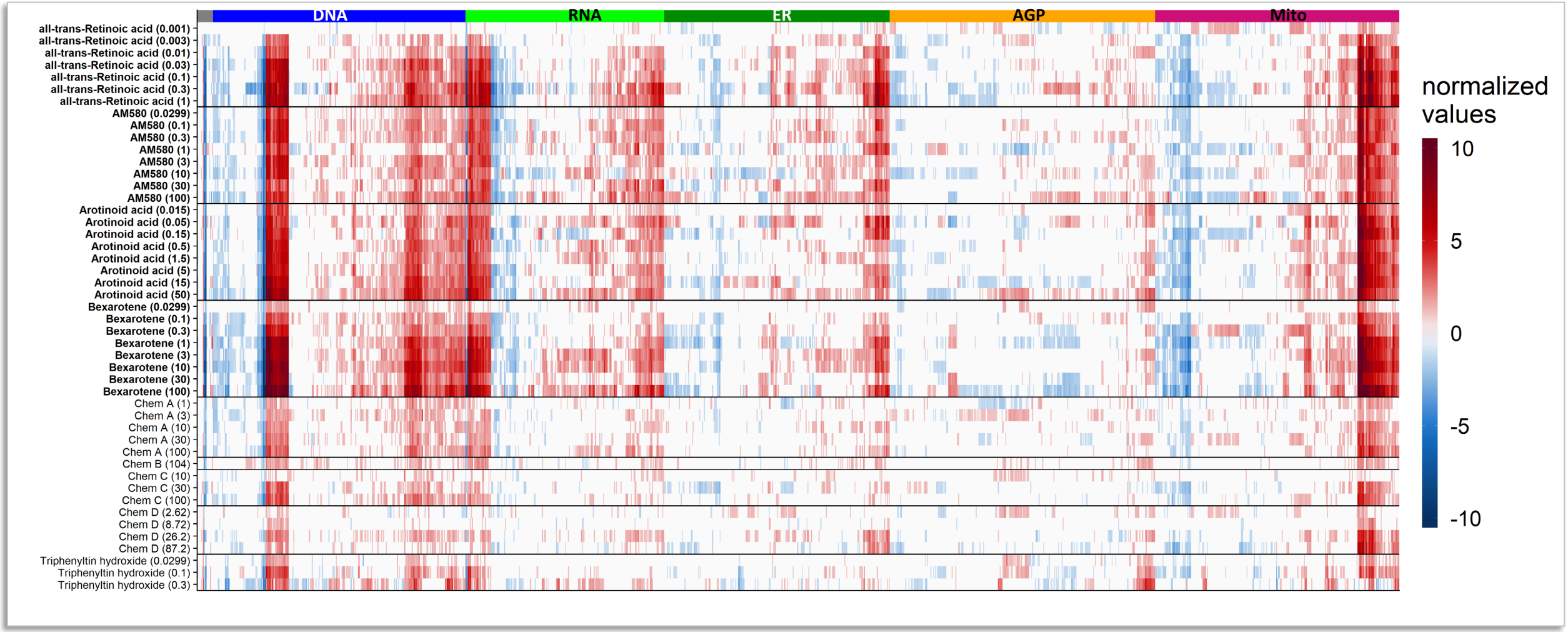
known retinoids





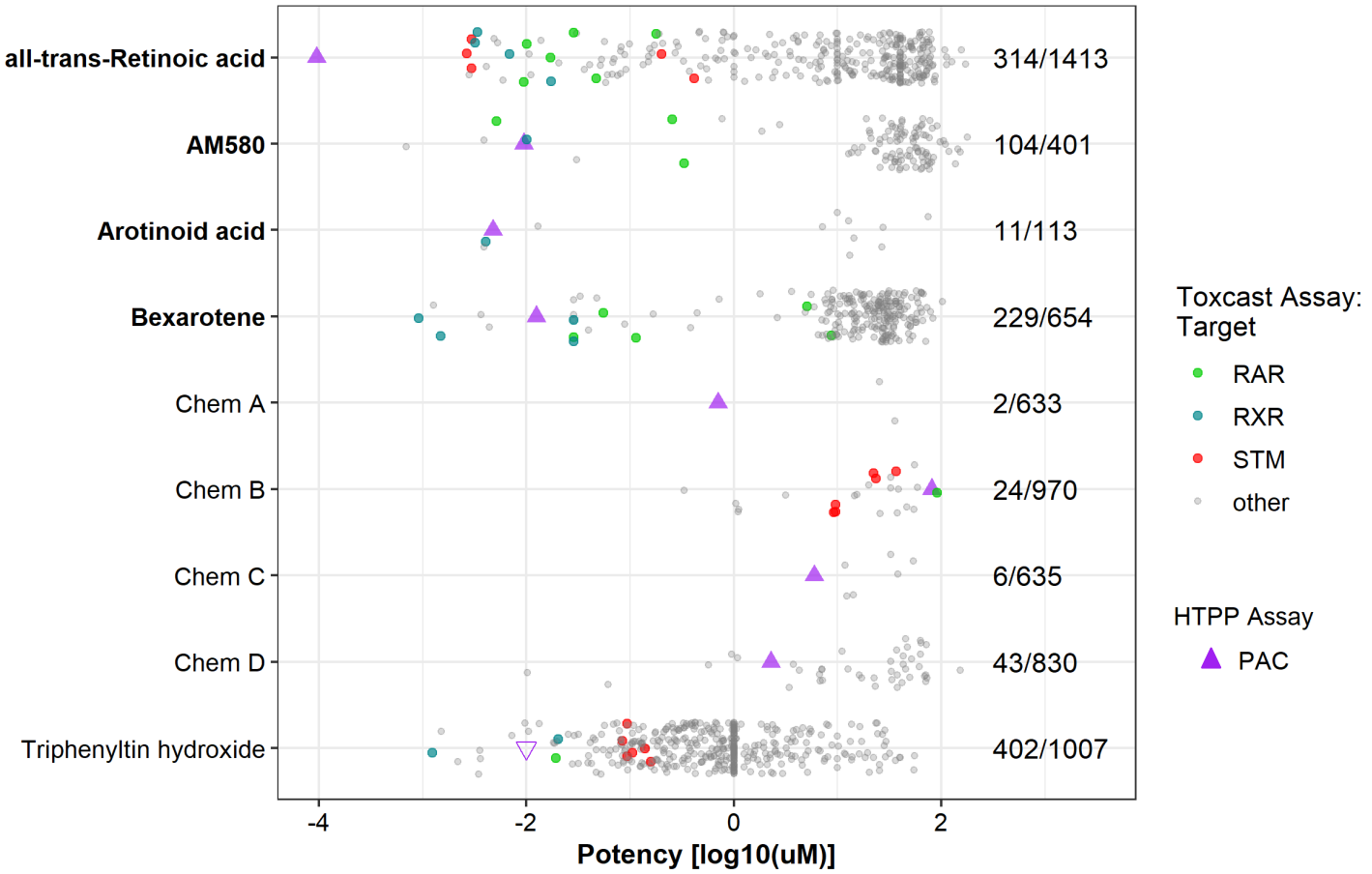
⇨ **These five chemicals were highly similar to the known retinoids but did not display similarity with other chemicals.**

Identify test chemicals with similar profiles (I)



⇨ **These five chemicals were highly similar to the known retinoids but did not display similarity with other chemicals.**

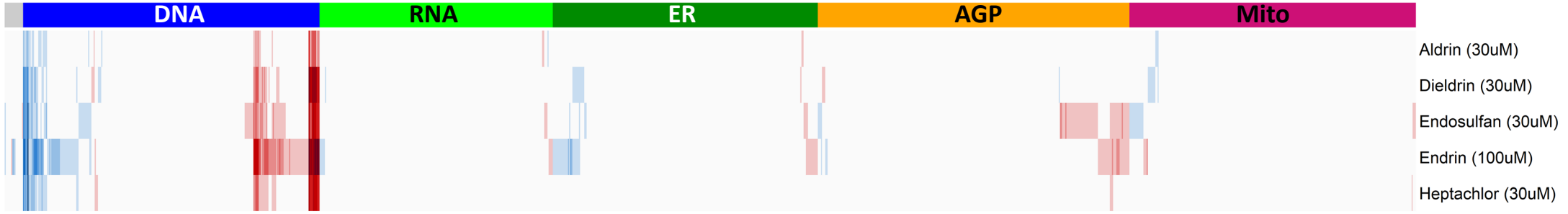
Identify test chemicals with similar profiles (II)



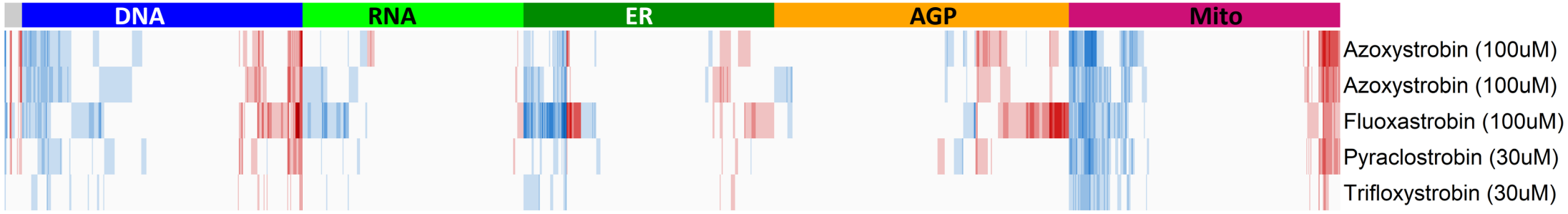
- ⇨ **4/5 test chemicals were not active in the ToxCast RAR and RXR assays**
- ⇨ **HTPP could yield complementary information to existing assays**

Non-drug like chemicals

Organochlorides:



Strobilurins:



⇨ **Certain groups of environmental chemicals display characteristic profiles**

Conclusion II

- Chemicals with different MOA display characteristic profiles (i.e. GR, RAR/RXR)
- We can identify test chemicals that are biologically similar to annotated chemicals (i.e. retinoids)
- Certain groups of environmental chemicals display characteristic profiles

HTPP can potentially be used to derive mode-of-action information and help in prioritization of lower tier follow up assays

Next steps:

- confirm the suspected retinoids in an orthogonal assay (qPCR) – ongoing
- How well does structural similarity translate into biological similarity?

Thank you for your attention!

Questions?

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