Phenotypic profiling to identify putative mechanisms of environmental chemicals

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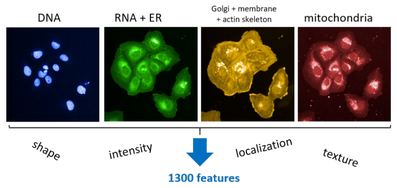
What is phenotypic profiling?



- Image-based phenotypic profiling is a chemical screening method that measures a large variety of morphological features of individual cells in *in vitro* cultures.
- No requirement for *a priori* knowledge of molecular targets.
- **May be used as an efficient and cost-effective method for evaluating chemical bioactivity.**

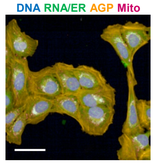
Goal

Investigate whether phenotypic profiling can be used to identify putative mechanism-of-action based on shared profiles among chemicals.

For this purpose, a set of 1201 chemicals (mostly from the ToxCast chemical library) were screened in the 'Cell Painting' assay. A subset of chemicals (n = 179) were annotated with a biological target.

Method: High-throughput phenotypic profiling (HTPP)

1. Chemical exposure & labeling ('Cell Painting')

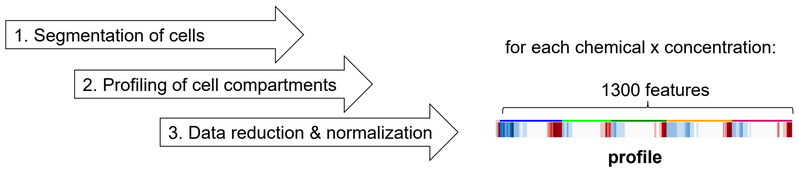


Experimental design	
Cell type	U-2 OS
Exposure time	24 h
Cell seeding density per well	3000
# unique chemicals	**1201**
# concentrations	8
Concentration spacing	½ $\log_{10}$
# solvent controls/plate	18
# replicates/plate	1
# independent experiments	4

Organelle/Structure	Label
DNA	H-33342
RNA	SYTO14
Endoplasmic reticulum (ER)	Concanavalin A-488
Actin	Phalloidin-568
Golgi + Membrane	Wheat germ agglutinin -555
Mitochondria	MitoTracker

For more experimental details, refer to Nyffeler et al. 2020; PMID: 31899216, DOI: 10.1016/j.taap.2019.114876

2. Generation of profiles



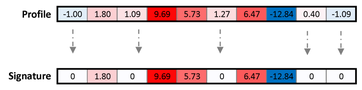
3. Calculation of biological similarity

1. Feature Selection

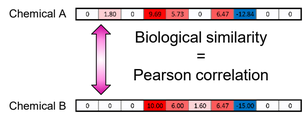
Only features with at least a correlation > 0.25 across biological replicates were retained: 824/1300 features

2. Generation of signatures

replacing |values| < 1.5 with 0



3. Comparison of signatures

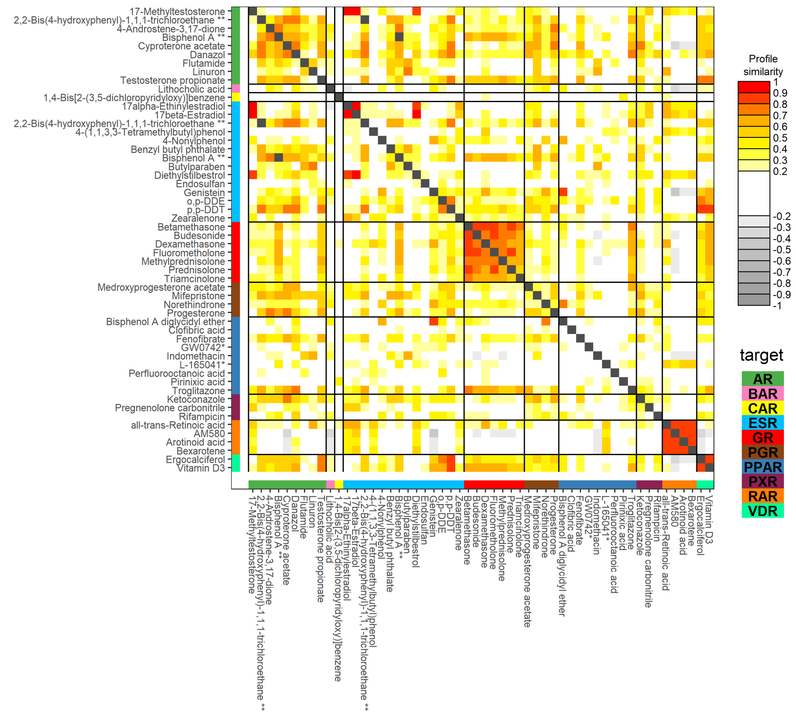


4. Summarizing

For each chemical pair, the highest similarity of all comparisons of the three lowest active concentrations is retained as the similarity score.

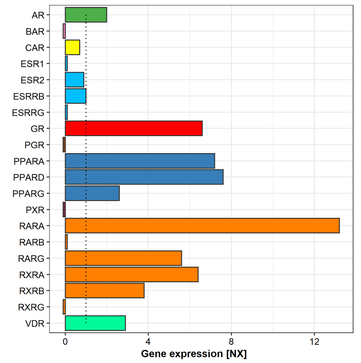
Biological similarity of annotated chemicals: Example of nuclear receptor modulators

52 chemicals were annotated as being a modulator (activator, inhibitor, or unknown direction) of a nuclear receptor. Upon testing in the HTPP assay, biological profiles were derived and compared pairwise using Pearson correlation:



⇨ **only chemicals targeting glucocorticoid receptor (GR), retinoic acid receptors (RAR/RXR) and vitamin D receptor (VDR) had characteristic profiles.**

Investigation of the gene expression levels in U-2 OS cells revealed that only some nuclear receptors were expressed:



Gene expression levels for U-2 OS cells were retrieved from the human protein atlas, www.proteinatlas.org). Genes with an NX value < 1 are considered not expressed (indicated by the vertical dotted line).

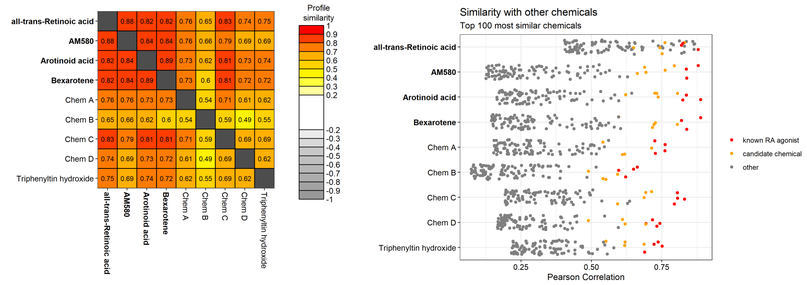
⇨ **The targets for which we observed characteristic profiles are expressed (GR, RAR, RXR, VDR)**

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

⇨ **For certain targets, reference chemicals produced a characteristic profile, suggesting that they have the same cellular effects**

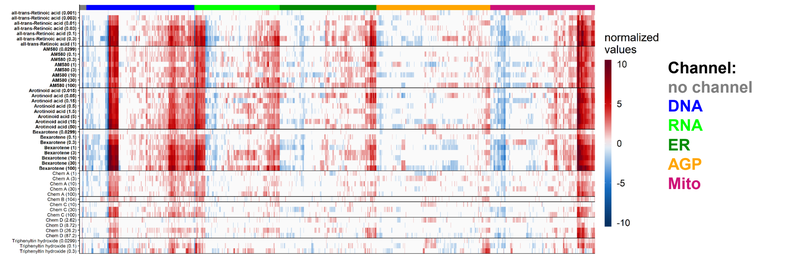
Biological similarity of test chemicals: Example of retinoids

Among all 1201 tested chemicals, five chemicals displayed high biological similarity to the known retinoids:



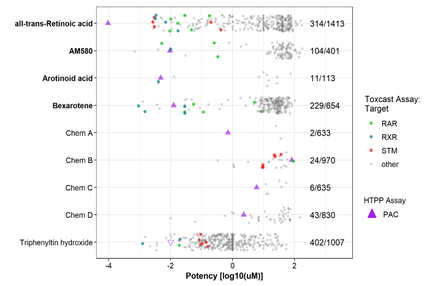
⇨ **These five chemicals were highly similar to the known retinoids but did not display similarity with other chemicals.**

Profiles for all retinoids and candidate chemicals (1300 features):



⇨ **The five chemicals have similar profiles albeit a weaker effect as known retinoids**

For these chemicals, all *in vitro* test results from the ToxCast assay suite were retrieved:



Potencies for ToxCast assays were retrieved from invitroDB v.3.3. The number of active assays out of total assays tested for each chemical is indicated on the right. For each active assay, a potency value is displayed. Color coding indicates the assays targeting the RAR/RXR pathway (green/turquoise) and a stem cell-based assay that is sensitive to retinoic acid pathway disturbance (STM, red). The potency estimated from the HTPP assay is indicated in purple triangles.

⇨ **Only one chemical (triphenyltin hydroxide) was active in the ToxCast RAR/RXR assays. It is a known RXR agonist.**

⇨ **One additional chemical was active in the STM assay**

⇨ **Three chemicals were tested in RAR/RXR/STM assays but were inactive**

⇨ **Five test chemicals had a similar phenotype as known retinoids**

Next steps:

- **Confirm the bioactivity of these chemicals in an orthogonal assay (qPCR)**

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