

Toxicogenomics with the zebrafish embryo model

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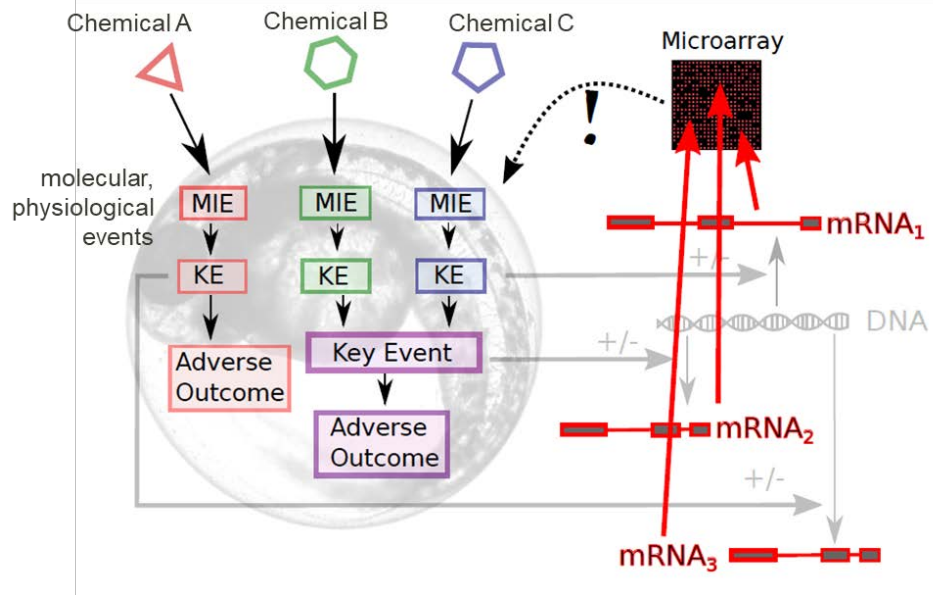
from individual observations to comprehensive mixture predictions

Dr. Wibke Busch

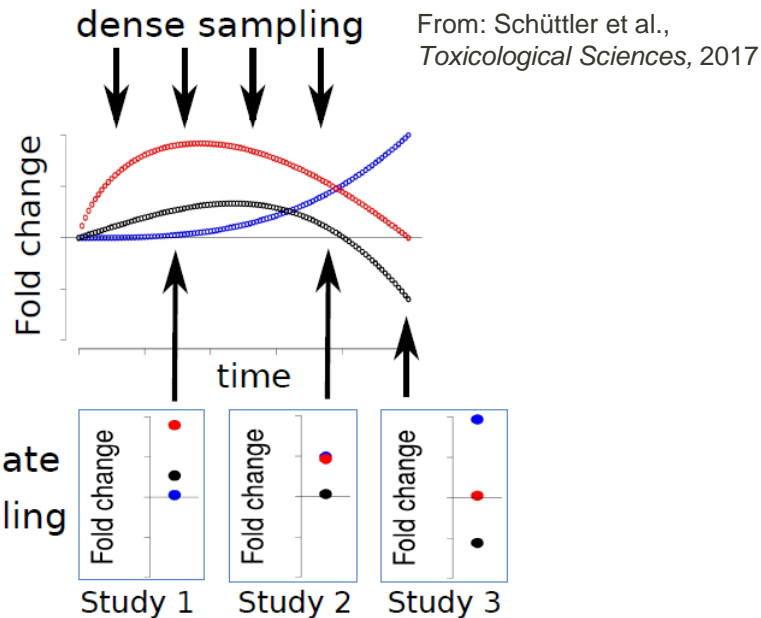
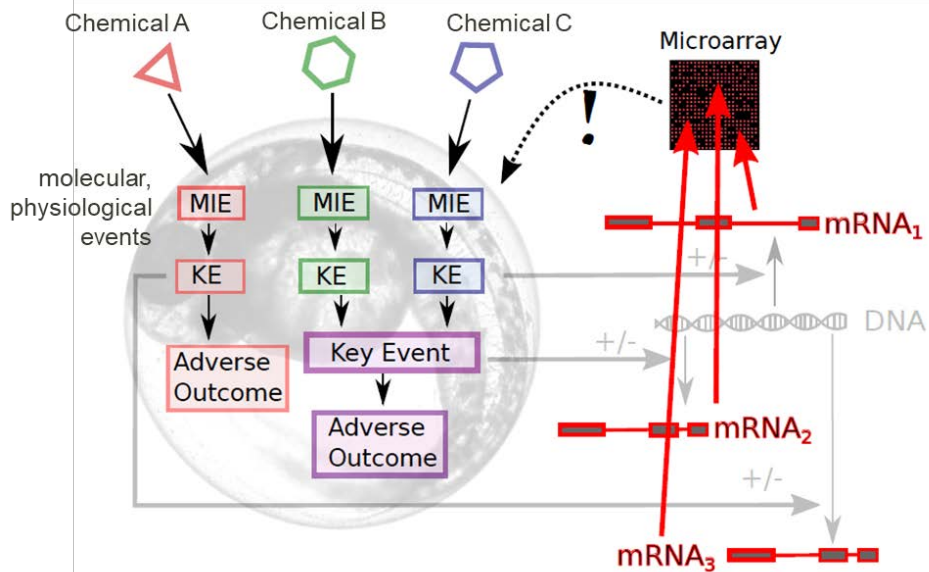
Head of integrative toxicology (iTox) group
Department Bioanalytical Ecotoxicology (BIOTOX)

November 2021

Toxicogenomics



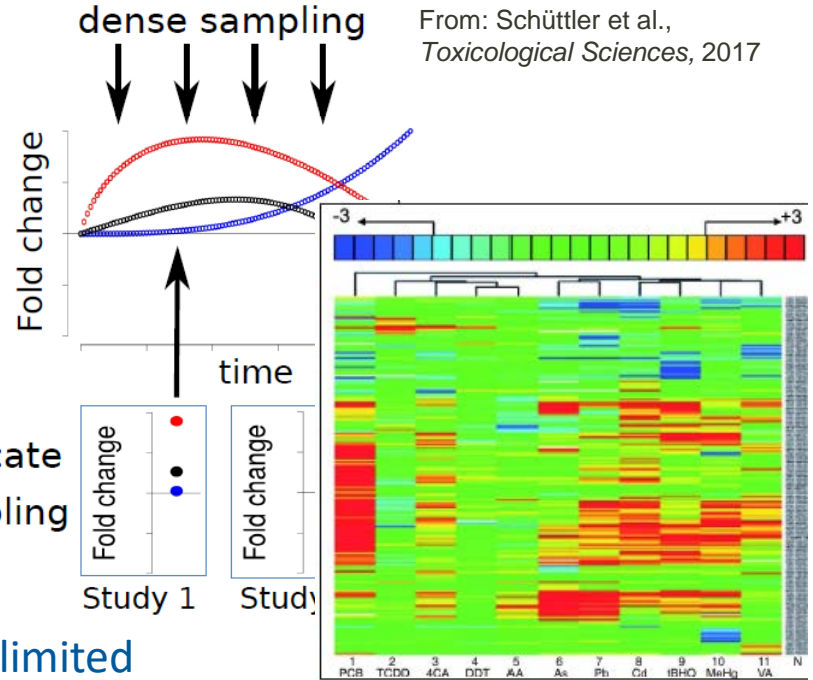
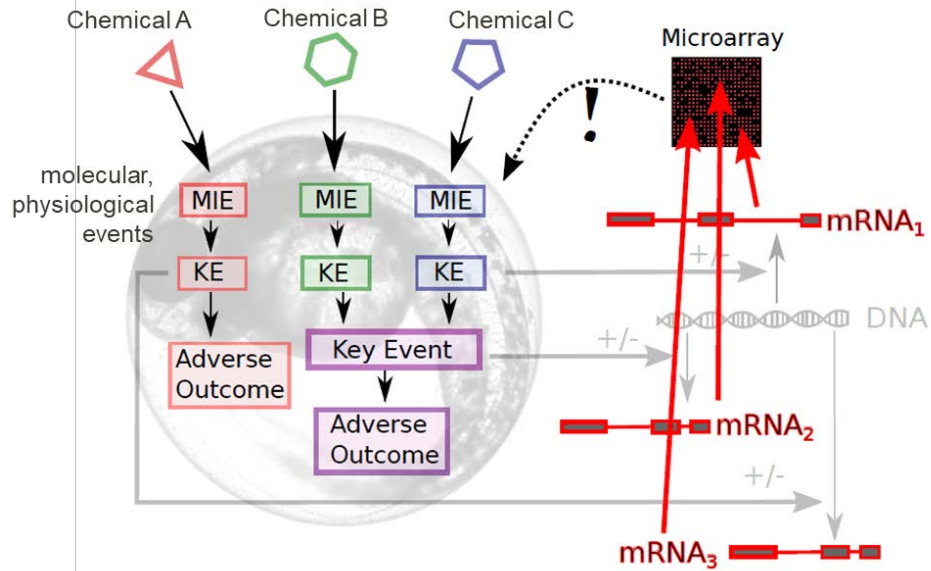
Toxicogenomics



→ Comparability between toxicogenomic findings is limited

- Time/Concentration dependency often neglected

Toxicogenomics



From: Schüttler et al.,
Toxicological Sciences, 2017

From: Yang et al., *Genome Biology*, 2007

→ Comparability between toxicogenomic findings is limited

- Time/Concentration dependency often neglected
- Commonly only subset of genes is reported/evaluated

→ Hypothesis and predictions for potential mixture effects almost impossible

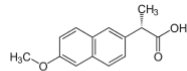
Questions and Approach

- How to see the complete picture?
- How to compare toxicogenomic effects of different substances?
- How to describe those effects mathematically to enable mixture calculations?

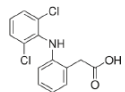
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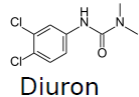
Experiment



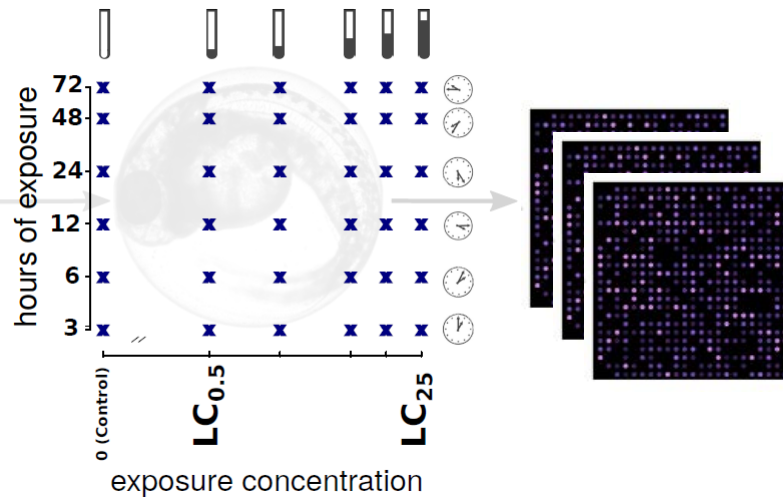
Naproxen



Diclofenac



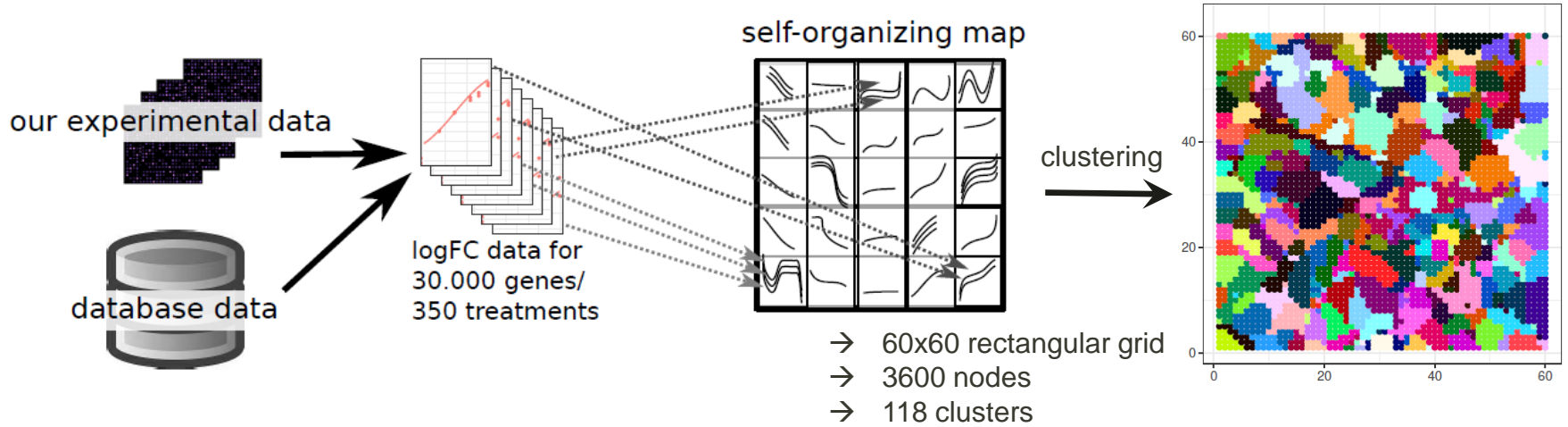
Diuron



Data analysis

- *integration* of previous data
- *aggregation* of fingerprints
- *modeling* of responses

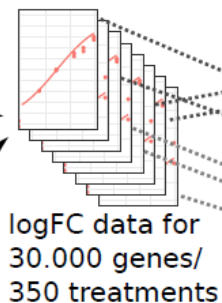
Integration and aggregation of data



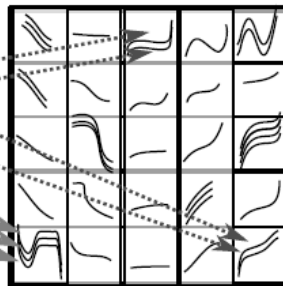
Andreas Schüttler et al.,
GigaScience, 2019

Integration and aggregation of data

our experimental data



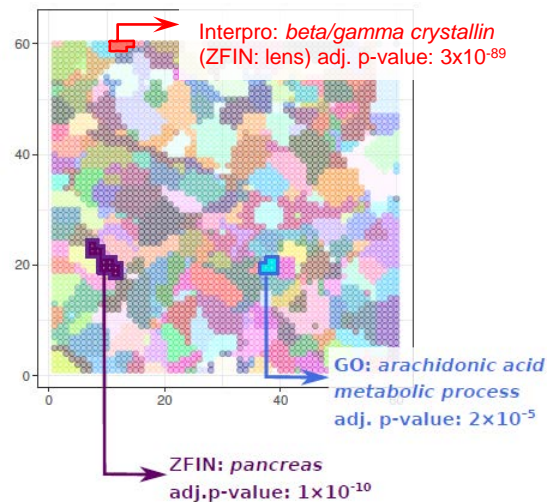
self-organizing map



clustering

- 60x60 rectangular grid
- 3600 nodes
- 118 clusters

functional enrichment



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GigaScience, 2019

Integration and aggregation of data

our experimental data

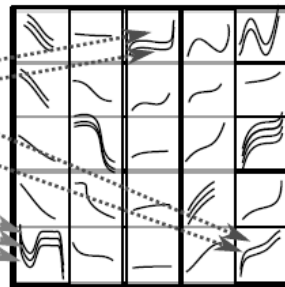
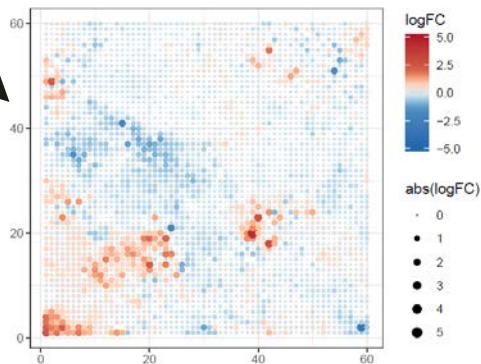
database data

logFC data for
30.000 genes/
350 treatments

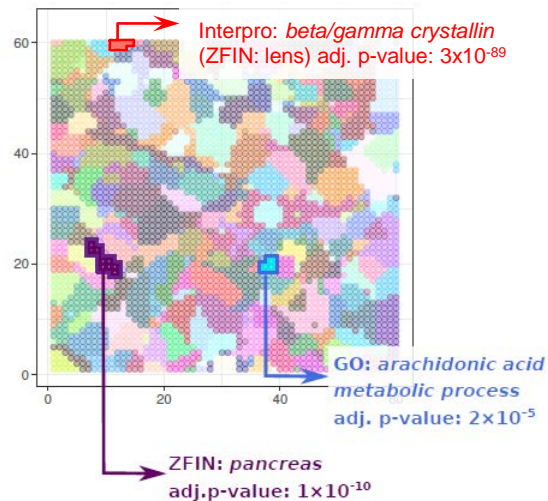
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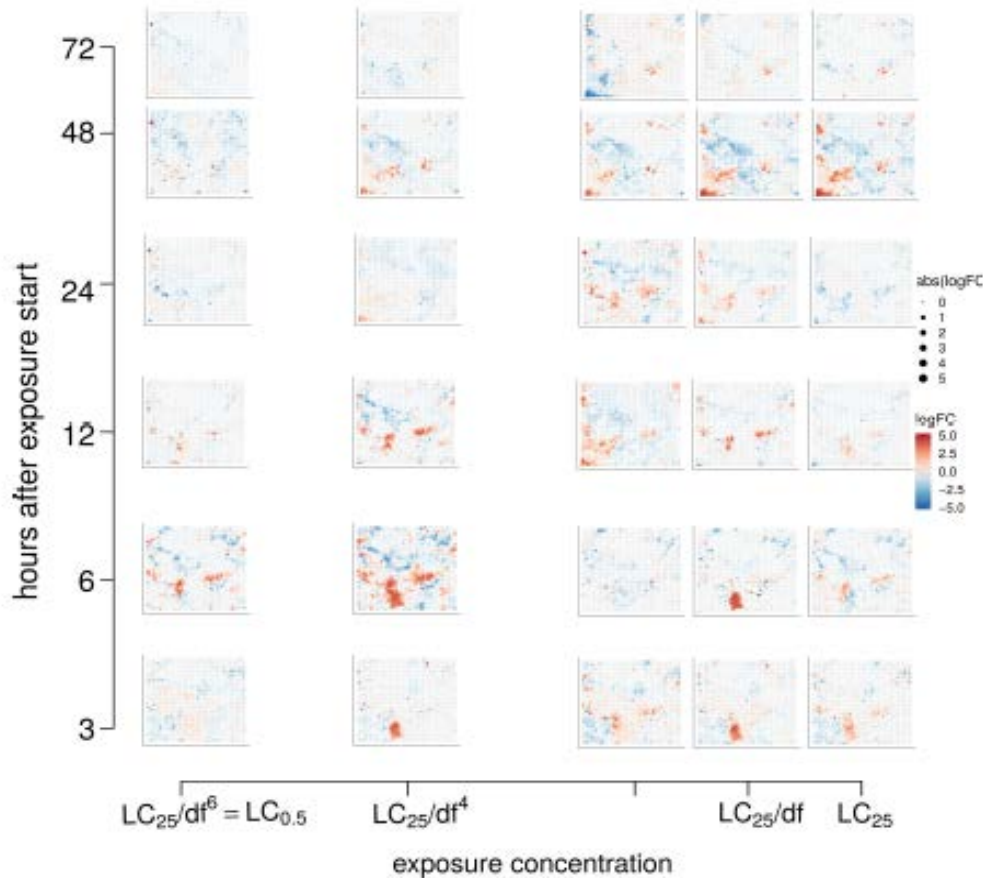
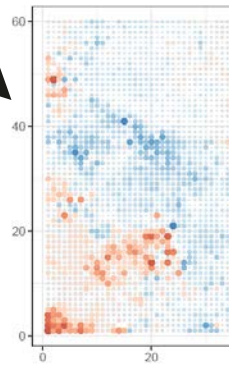
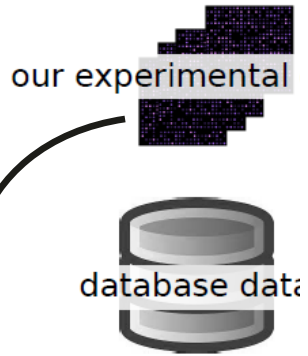


Diclofenac, 48 hours exposure, 7.4 μ M

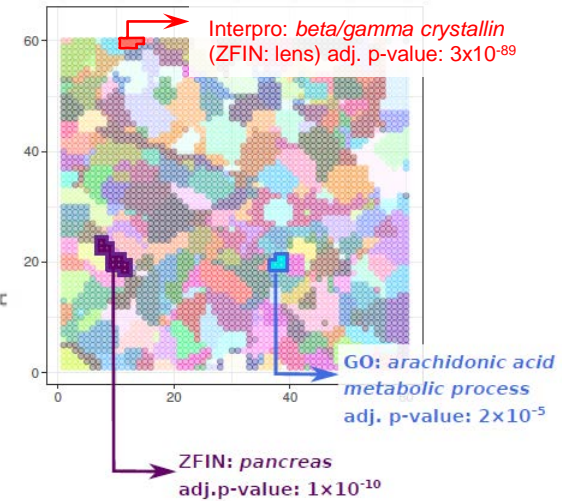


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Integration and aggregation of data

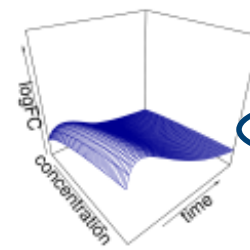
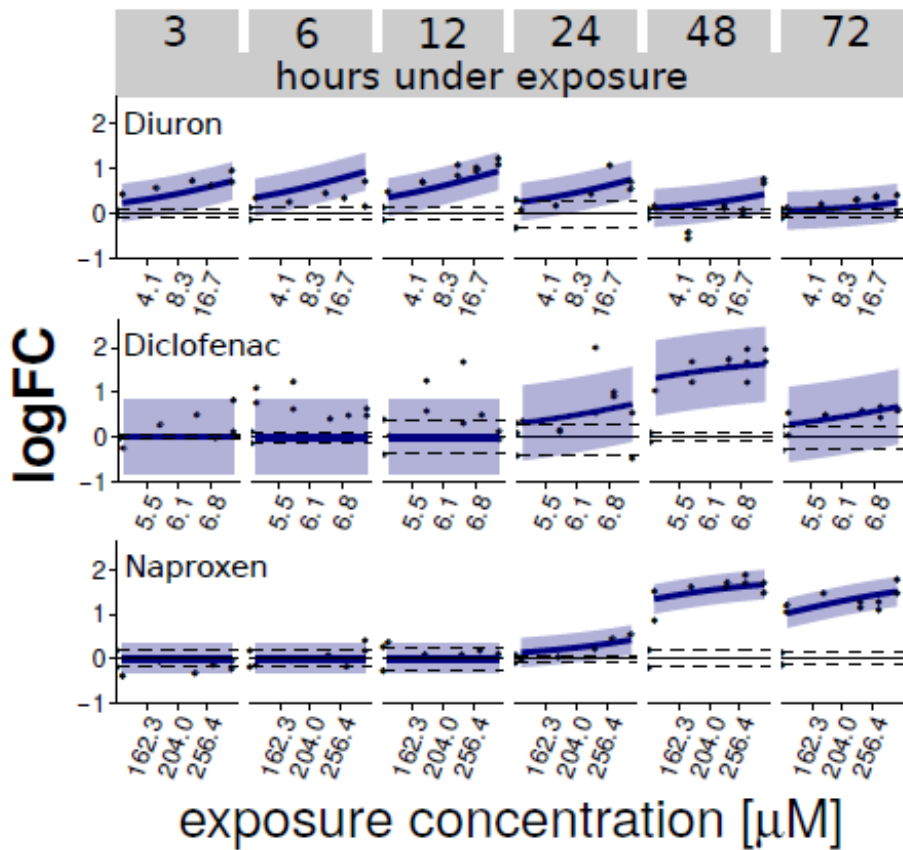


functional enrichment

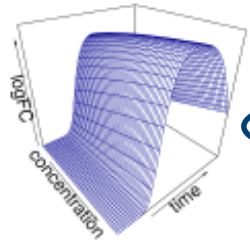


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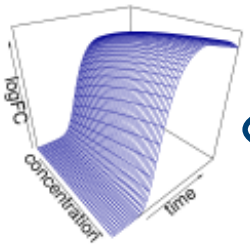
Regression modeling



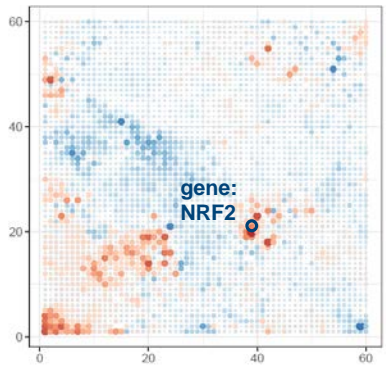
$S_{max}: 0.041$
 $S_{dur}: 0.8$
 $t_{max}: 8.8$
 slope: 0.6
 $\sigma: 0.3$



$S_{max}: 0.283$
 $S_{dur}: 0.42$
 $t_{max}: 41.3$
 slope: 3.2
 $\sigma: 0.5$



$S_{max}: 0.014$
 $S_{dur}: 0.35$
 $t_{max}: 50.6$
 slope: 1.6
 $\sigma: 0.2$

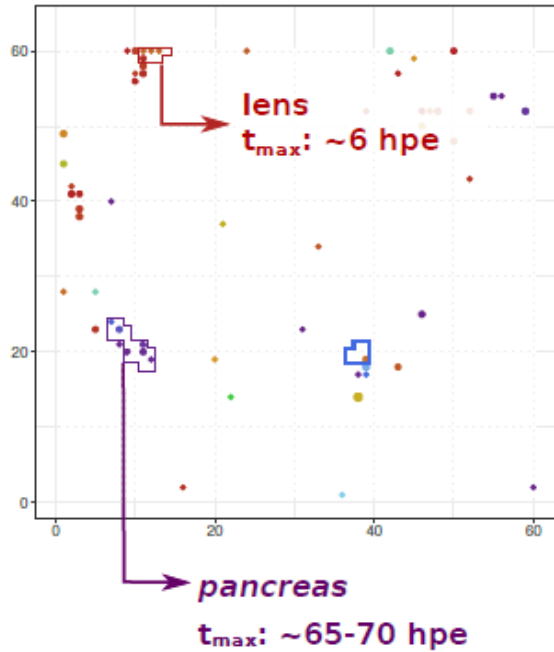


Mapping model parameters

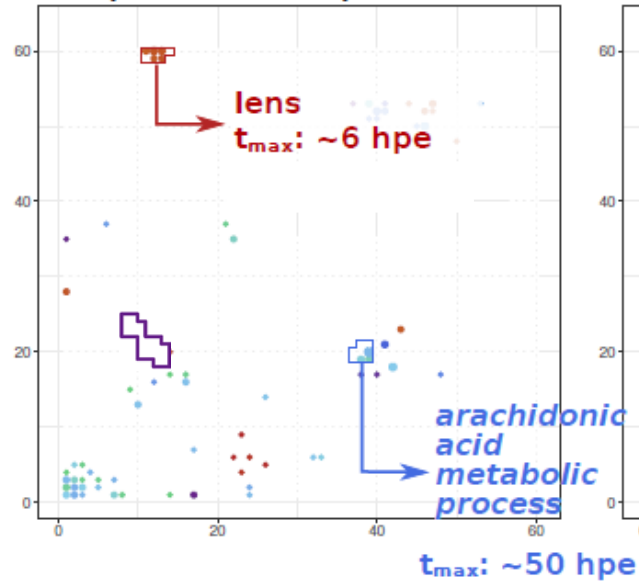
Herbicide (PSII Inhibitor)

Pharmaceuticals (Cox-inhibitors)

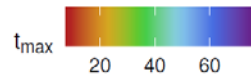
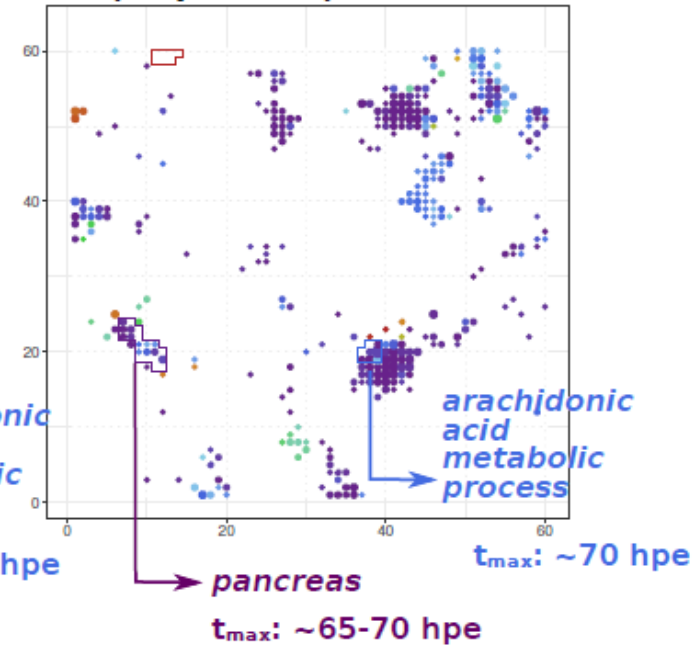
A (diuron)



B (diclofenac)



C (naproxen)



Adapted from: Schüttler et al., *GigaScience*, 2019

Mixture effects on the transcriptome

- Recovery of single substance effects?
- Occurrence of combined effects and relation to similar and dissimilar action?
- Can we predict the effects of a mixture?

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~ 30.000 genes → 3600 nodes of the map

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Goal: As many nodes as possible where more than one compound contributes to the potential effect of the mixture exposure

Mixture design

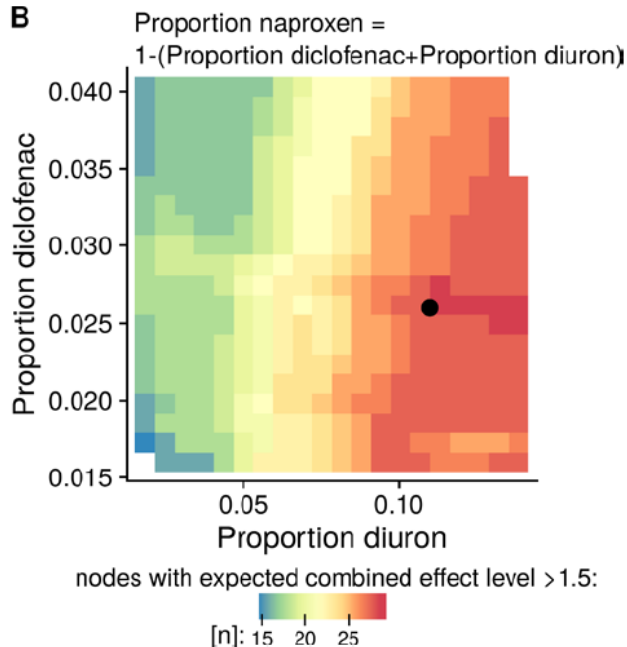
Concentration addition (CA) concept

CA calculations for:

ALL possible combinations of **component proportions**
for each node and each time point

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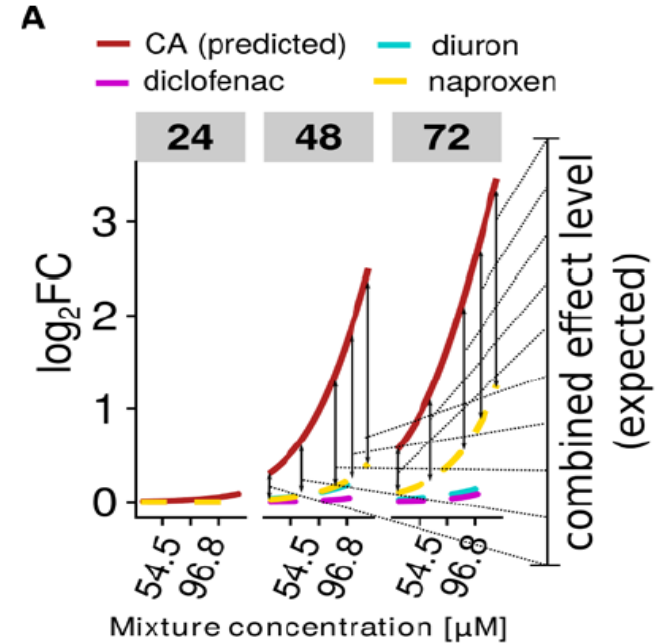
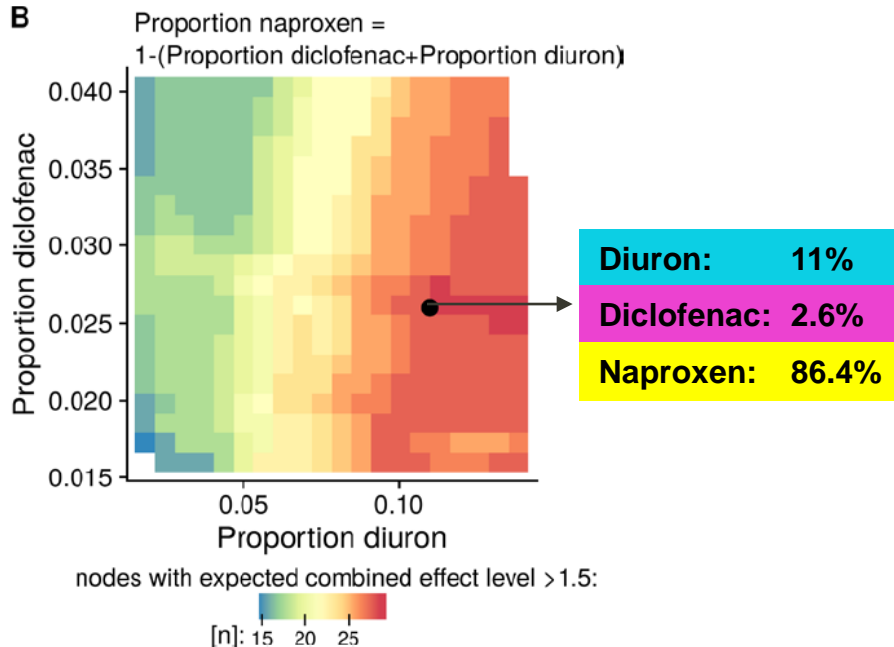


Mixture design

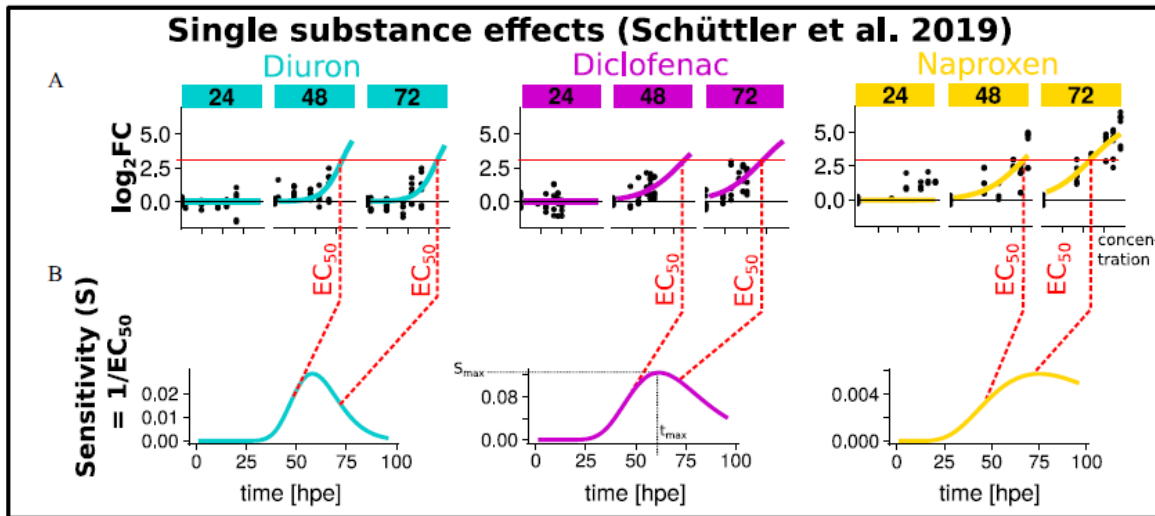
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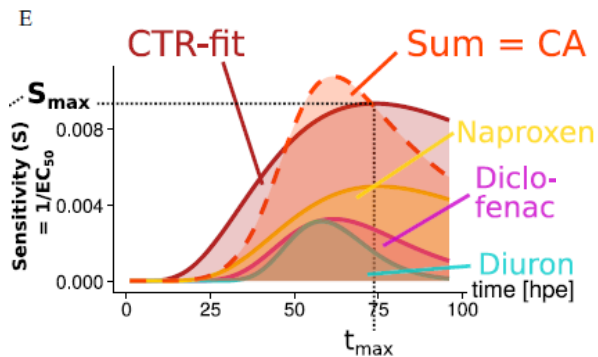


Mixture effect prediction



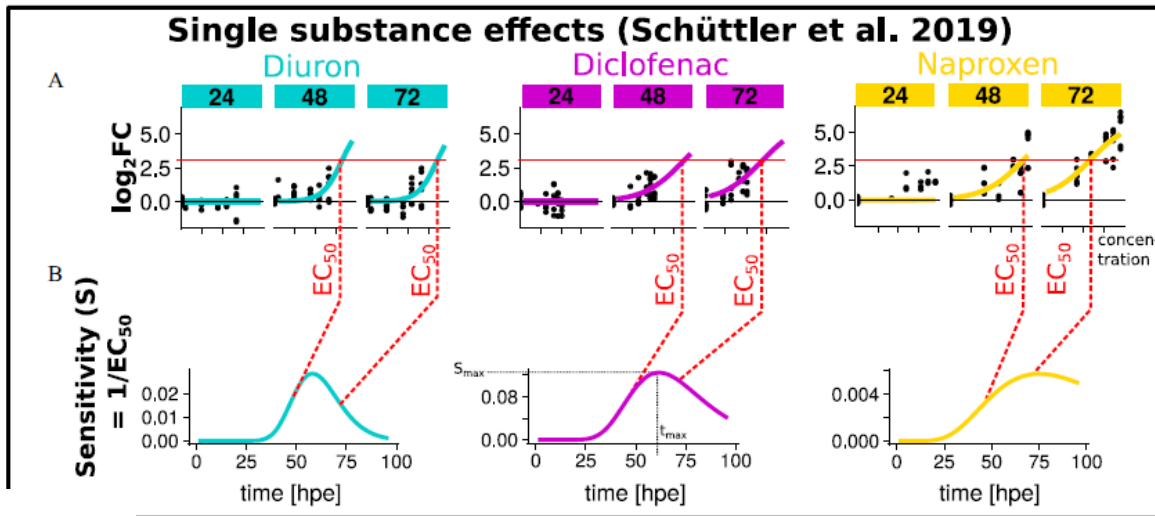
C Mixture proportions 11.0% 2.6% 86.4%

Mixture prediction - Concentration Addition (CA)

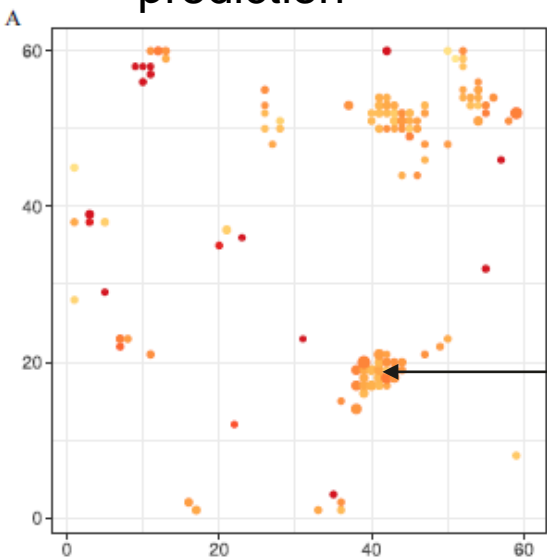


Mixture effect prediction

S_{max} map – prediction

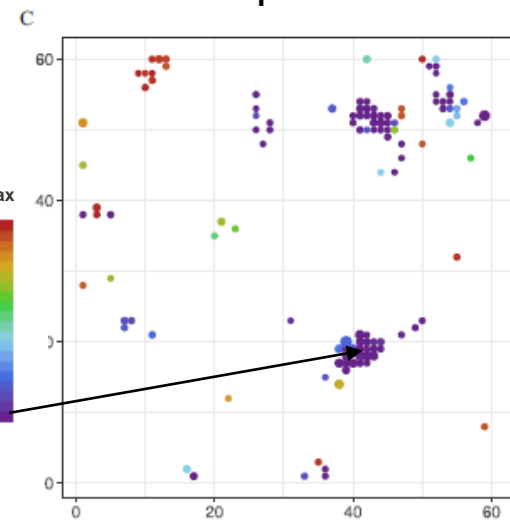
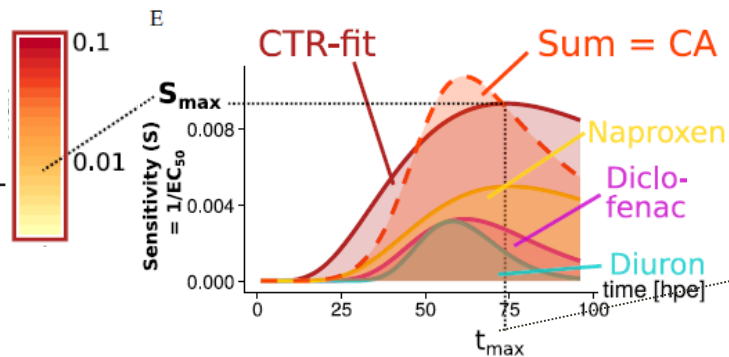


t_{max} map – prediction



reactions 11.0% 2.6%

Mixture prediction - Concentration Additive



Mixture effect prediction

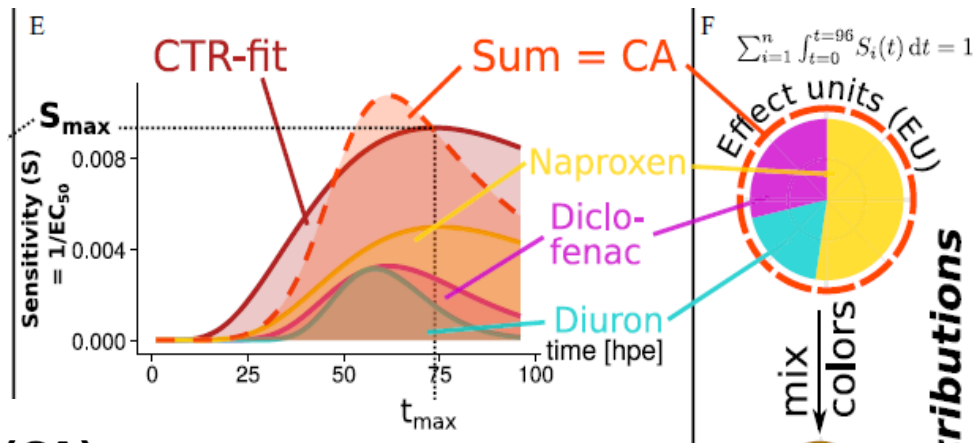
Predicted

to be significantly affected
(with CA concept)

= **122 nodes**

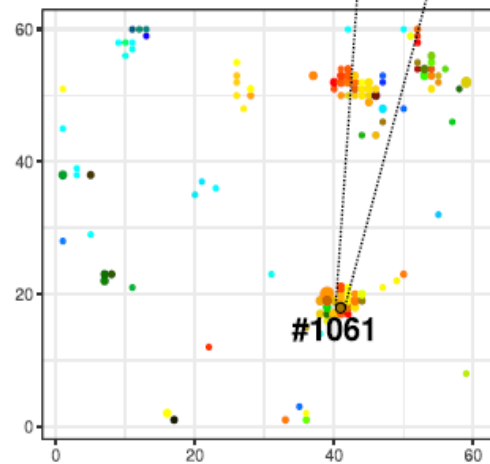
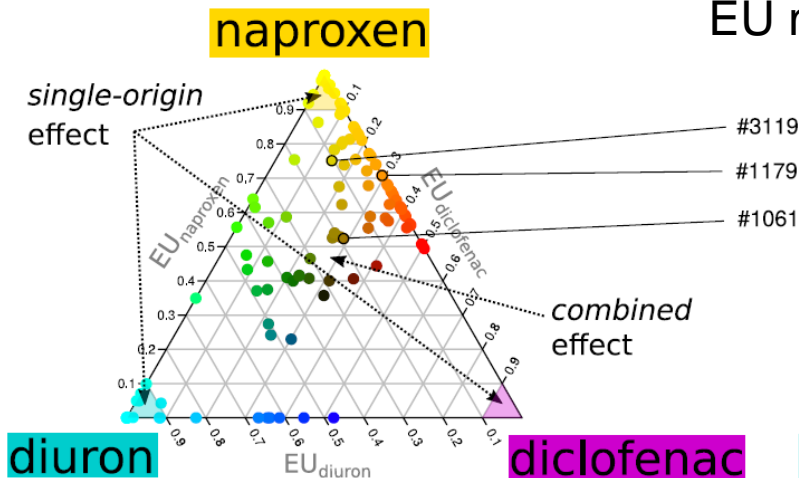
Mixture effect prediction

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Prediction (CA)

EU map - prediction



Components contributions

Mixture effect measurement

Observed

to be significantly affected

= **160 nodes**

- Exposure of ZFE to Mixture
- Microarray Measurements
- Data analysis - Fit CTR model
- Project model parameters on the map

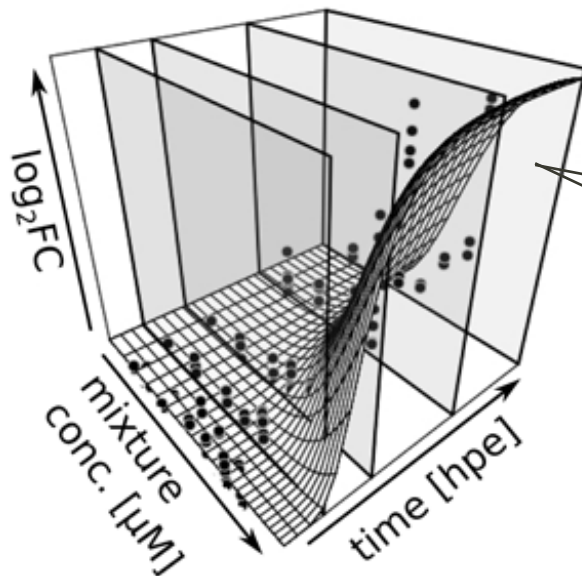
Mixture effect measurement

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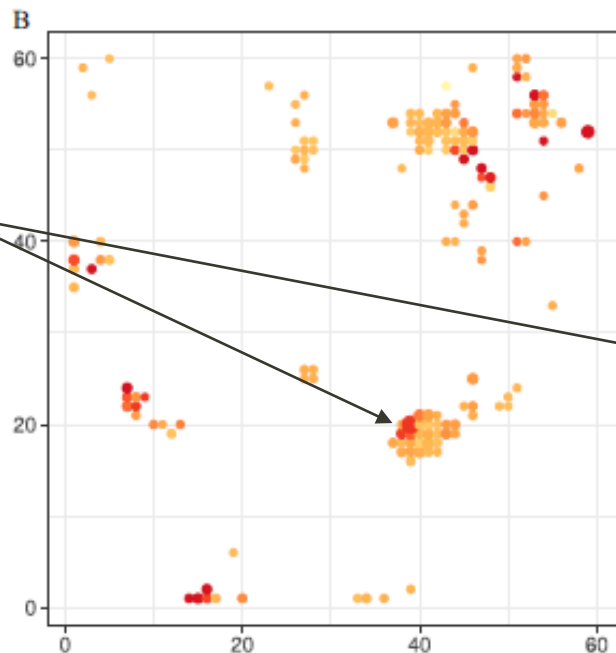
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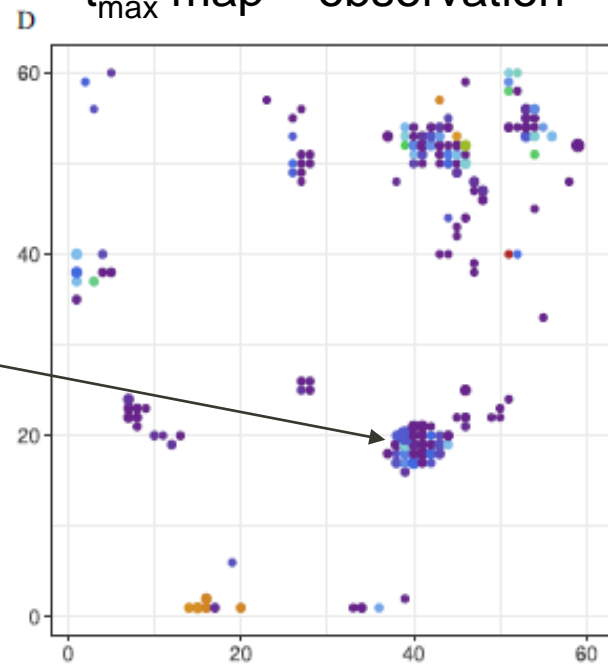
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S_{\max} map – observation

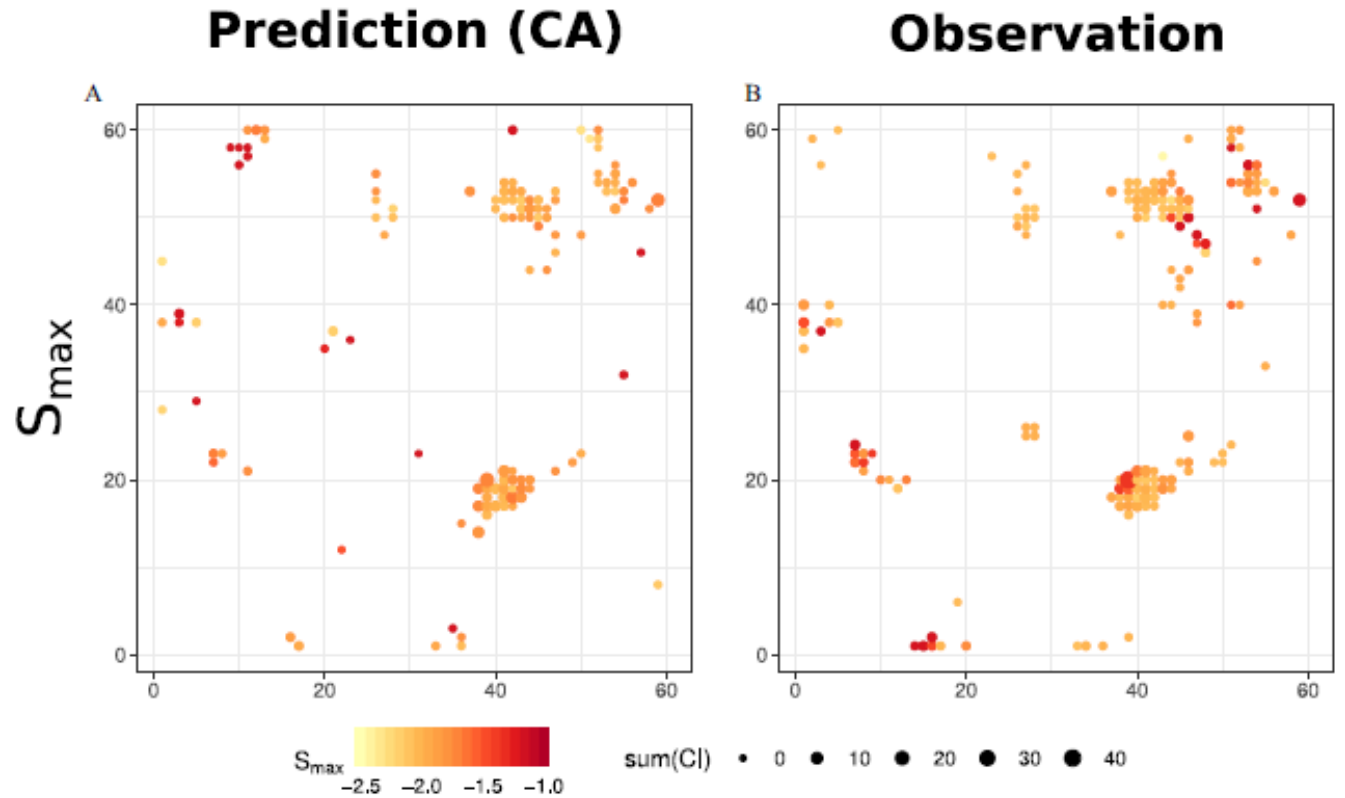


t_{\max} map – observation



Prediction vs Observation

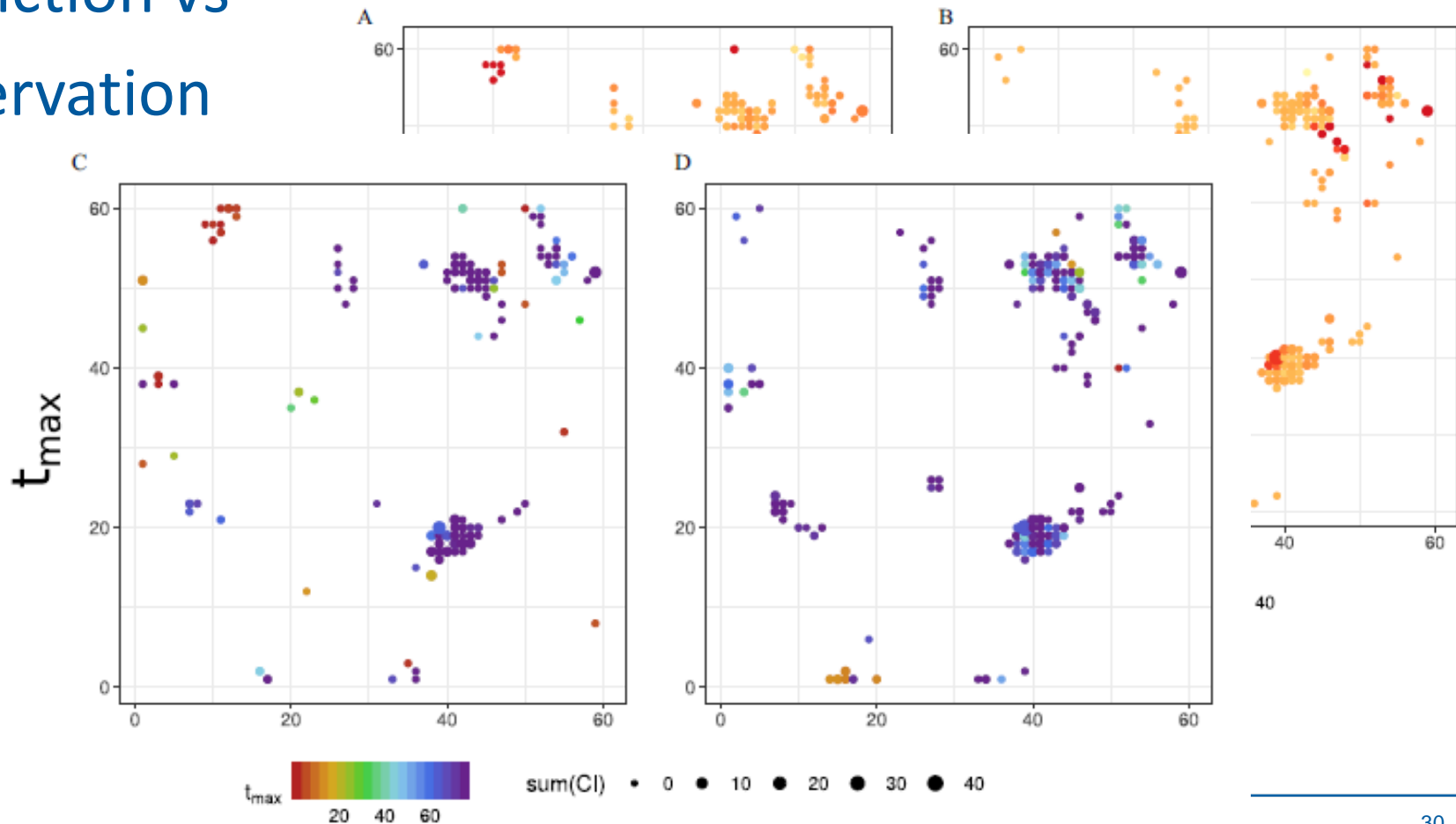
Prediction vs Observation



Prediction vs Observation

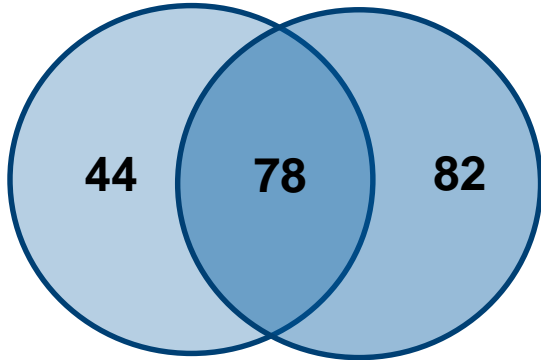
Prediction (CA)

Observation



Prediction vs Observation

Significantly affected

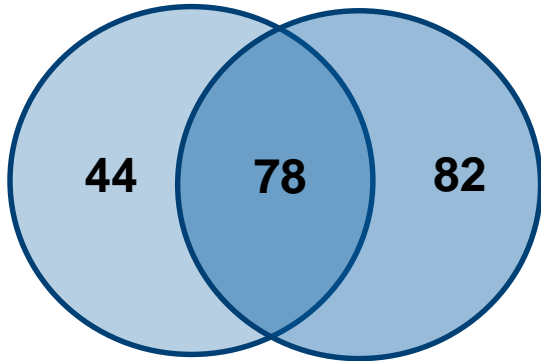


Predicted to **Observed to**
be affected: be affected:
122 nodes **160 nodes**

Total: **204 node**

Prediction vs Observation

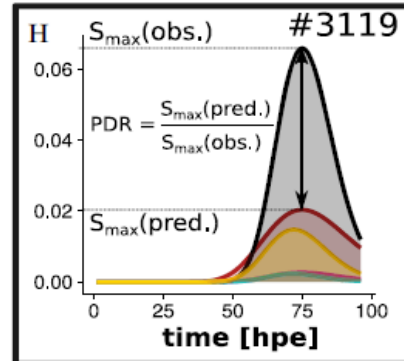
Significantly affected



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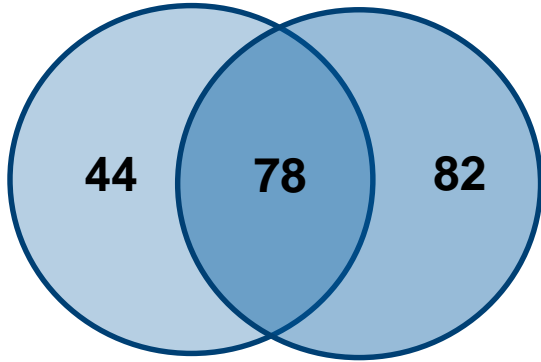
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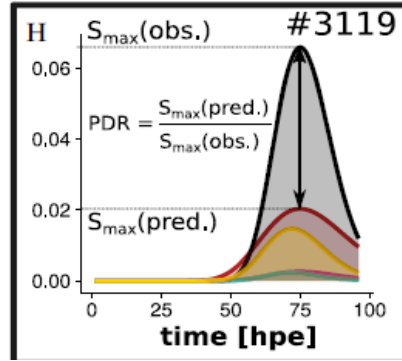
Significantly affected



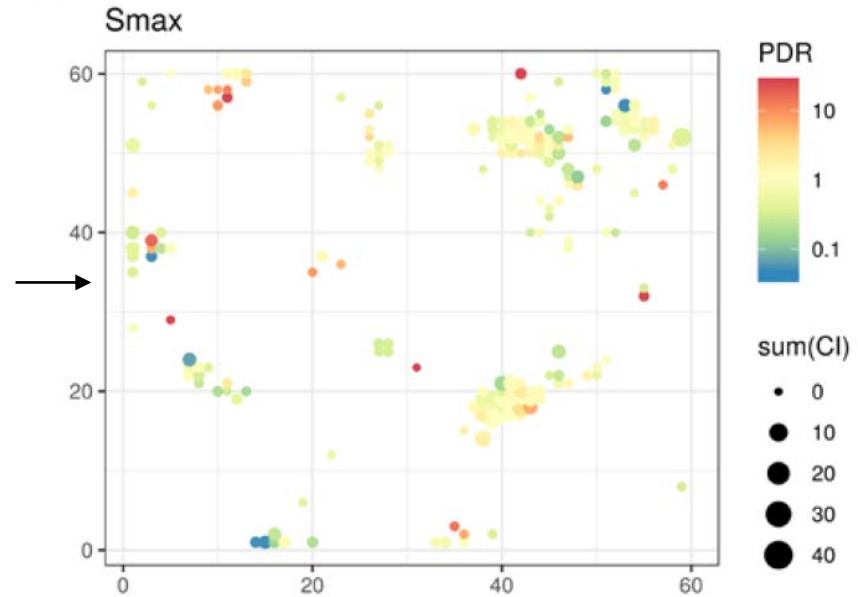
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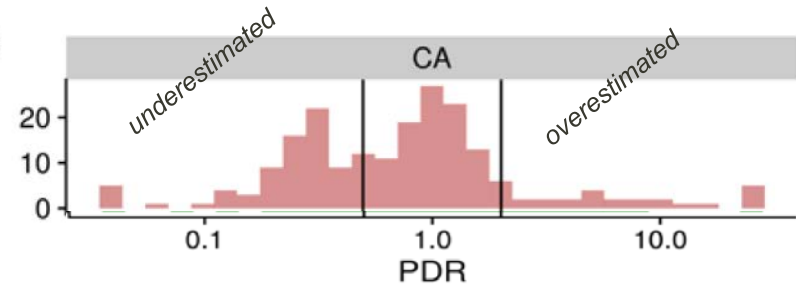
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A

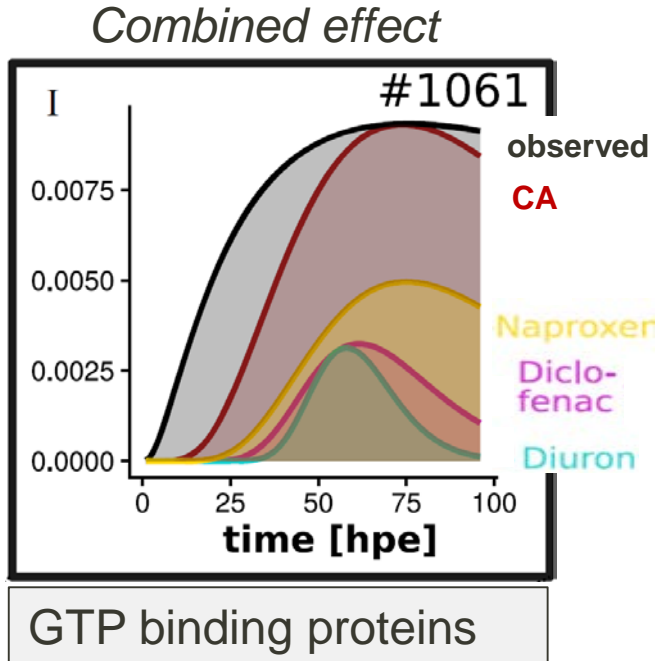


C



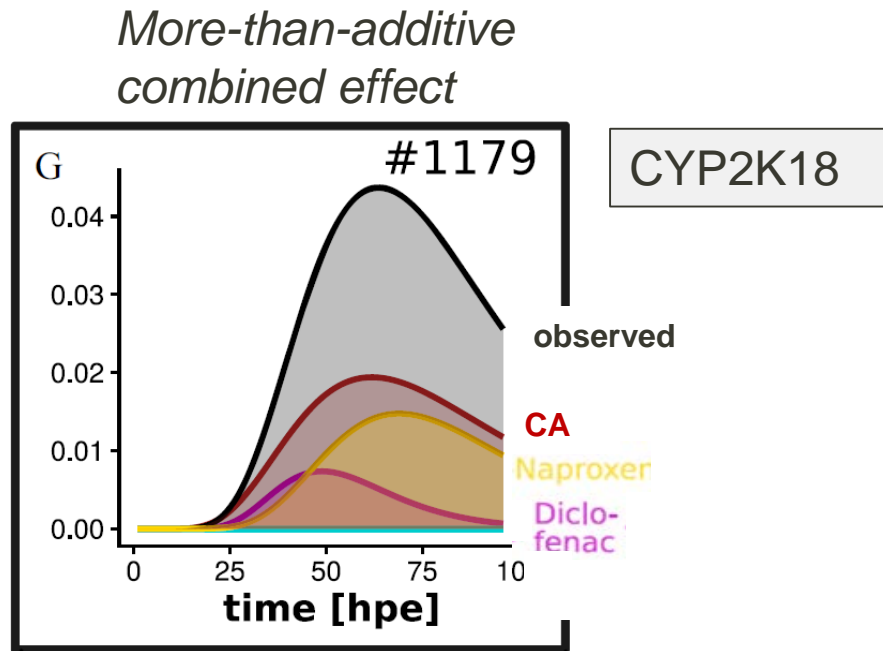
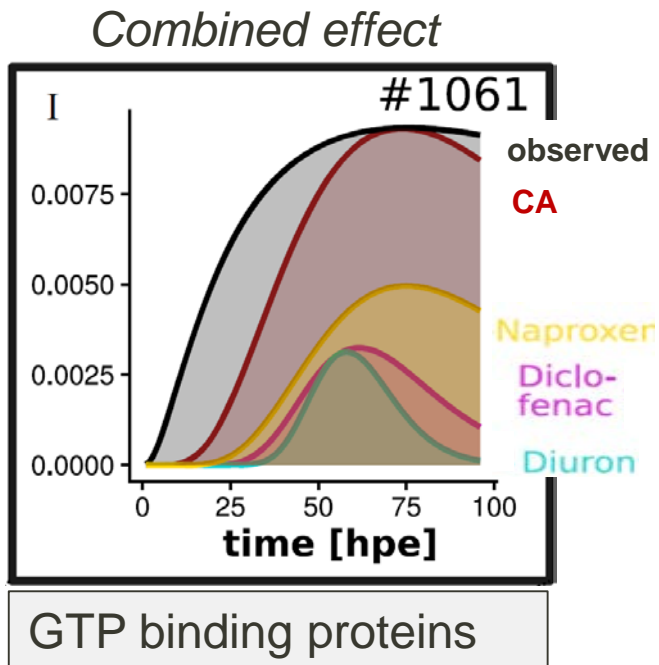
Deviations from Predictions

More-than-additive effects (more than one compound contributes to the combined effect AND observation > prediction):



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More-than-additive effects (more than one compound contributes to the combined effect AND observation > prediction):



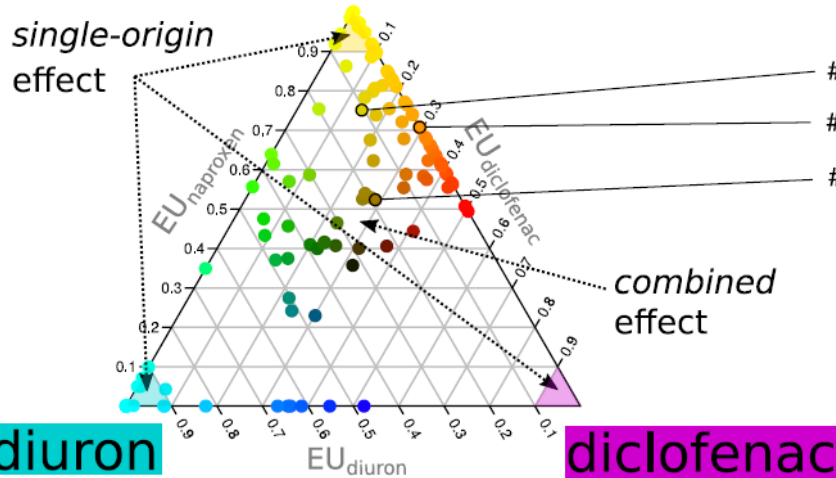
Prediction vs Observation - Component contributions

122 nodes

Prediction (CA)

naproxen

single-origin effect

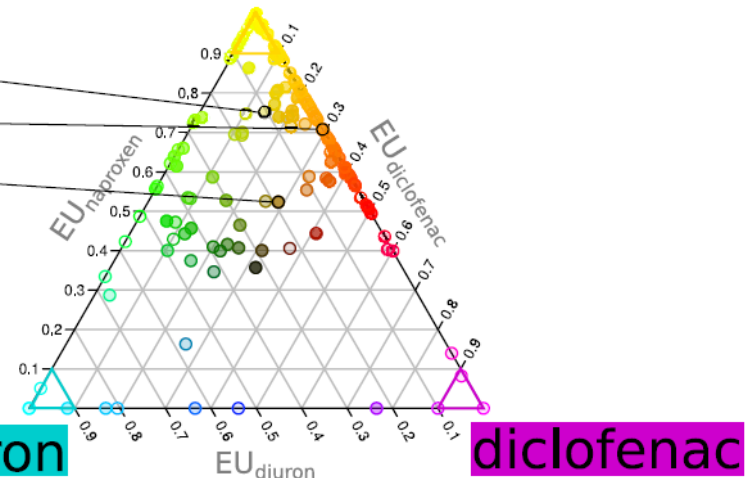


160 nodes

Observation

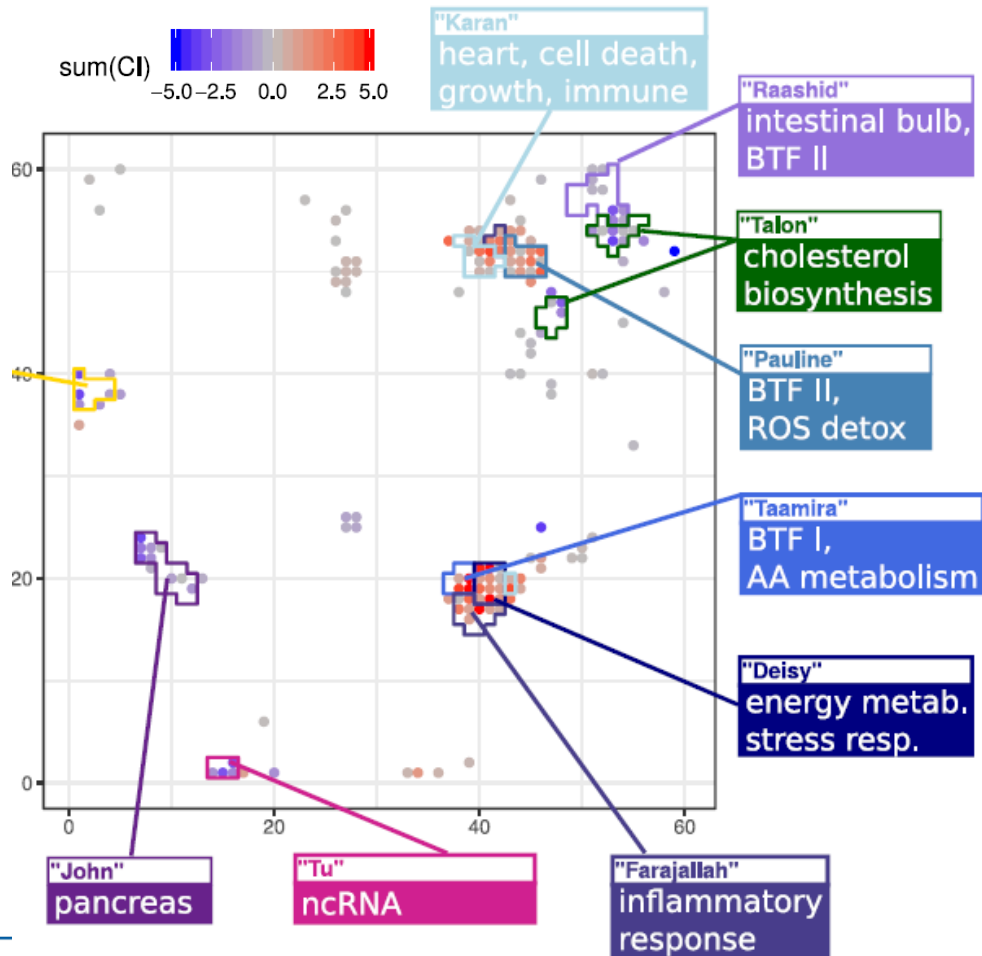
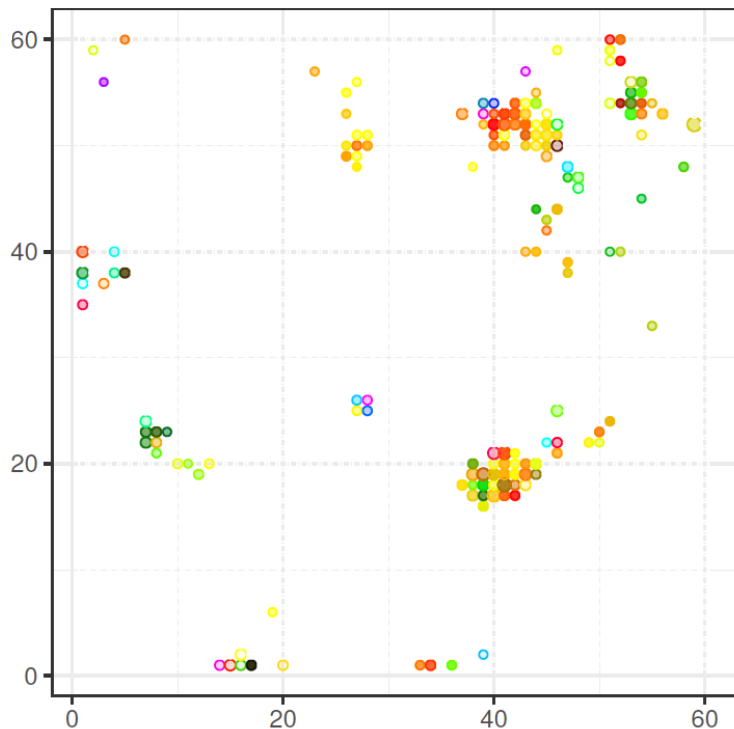
naproxen

B



Joint action of similar and dissimilar compounds

EU-map - Observed nodes



Summary | Conclusion

- Recovery of single substance effects? **YES**
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→ **Proof of concept:** molecular effects are not random and can be predicted for mixtures comprehensively based on individual component knowledge



Andreas Schüttler

Computational support:

UFZ WKDV: EVE, WOMBAT Team
(T. Schnicke, B. Langenberg, C. Krause, S. Petruschke, M. Abbrent, M. Garbe, N. Ziegner)

Bioinformatics group (M. Bernt, J. Hackermüller)

UFZ BIOTOX:
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Janet Krüger
Gianina Jakobs
Jelena Fix
David Leuthold
Nicole Schweiger
Silke Aulhorn

UFZ WANA:
Marin Krauss

Fraunhofer IZI
Kristin Reiche
Jörn Wiegand

Acknowledgement

Funding



Further information

- Schüttler et al. *Environmental Health Perspectives* 129(4), April 2021, <https://doi.org/10.1289/EHP7773>
- Looking into the nodes via our fingerprint browser: <https://webapp.ufz.de/itox/tfpbrowser/>
- Map and Model: Schüttler et al. *GigaScience* 8 (6), June 2019, <https://doi.org/10.1093/gigascience/giz057>