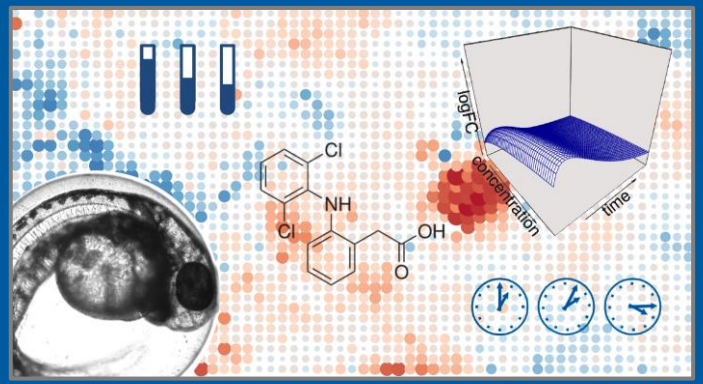
UFZ HELMHOLTZ Centre for Environmental Research

iTOX integrative toxicology

Combined Effects of Chemical Mixtures Are Predictable for the Whole Transcriptome – a Proof of Concept Study With Zebrafish Embryos

SOT
Joint RASS - MixSS
Webinar

14 September 2022



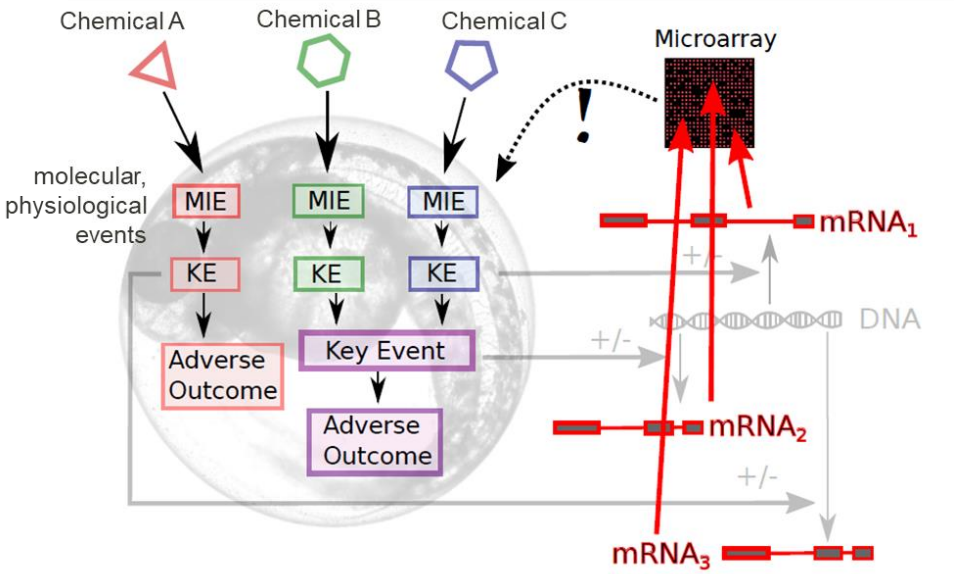
Graphics by A. Schüttler

Wibke Busch, PhD

Group leader iTOX,
Department Bioanalytical Ecotoxicology,
UFZ, Leipzig, Germany

Contact: wibke.busch@ufz.de

Toxicogenomics



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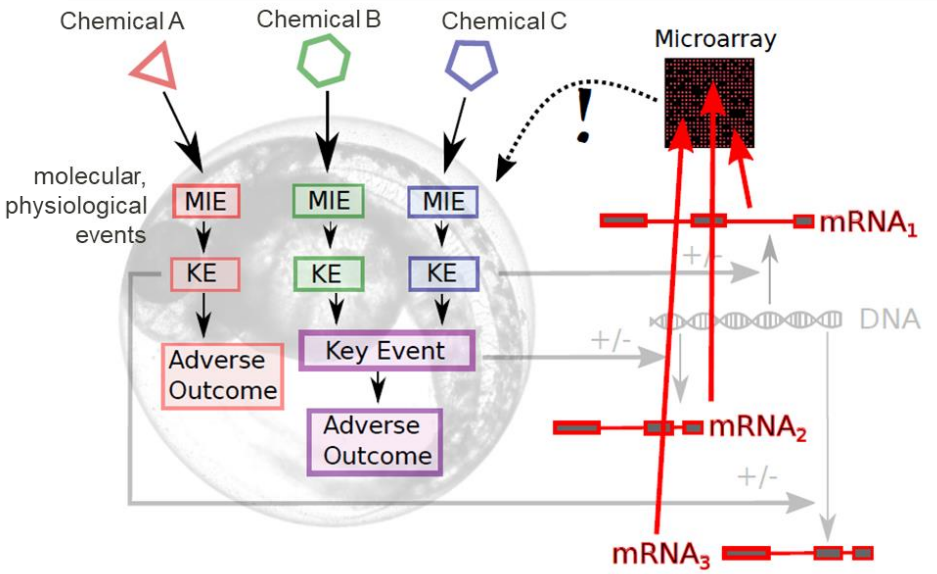

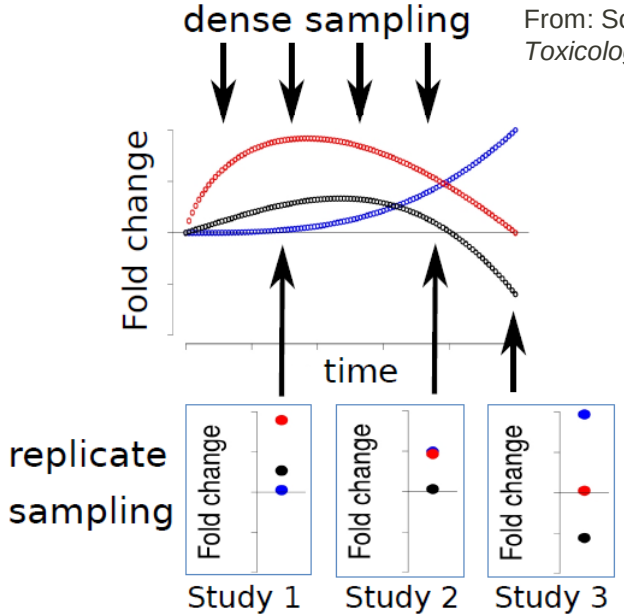

From: Schüttler et al., *Toxicological Sciences*, 2017

→ Comparability between toxicogenomic findings is limited

- Time/Concentration dependency often neglected

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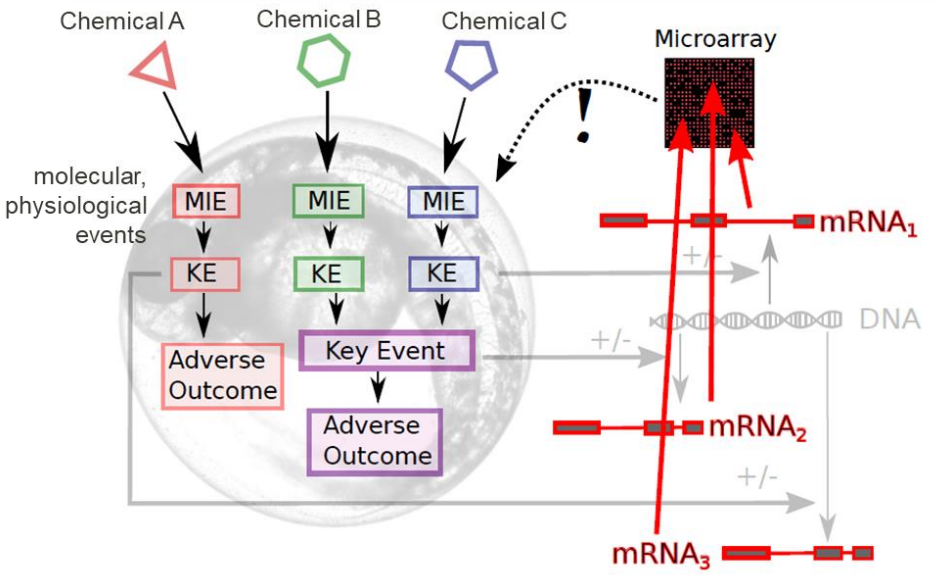

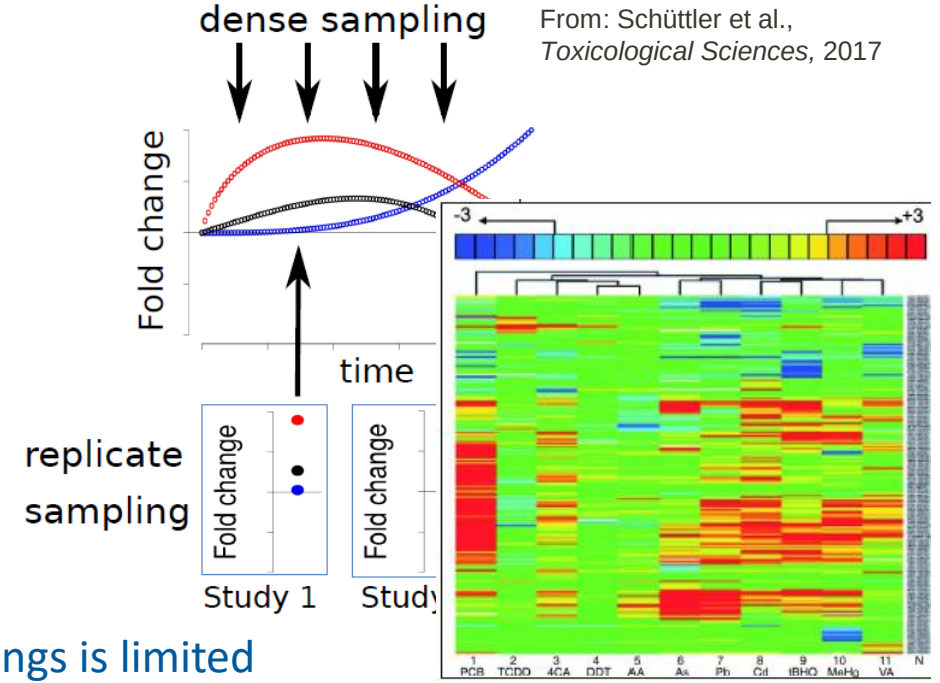

From: Schüttler et al., *Toxicological Sciences*, 2017

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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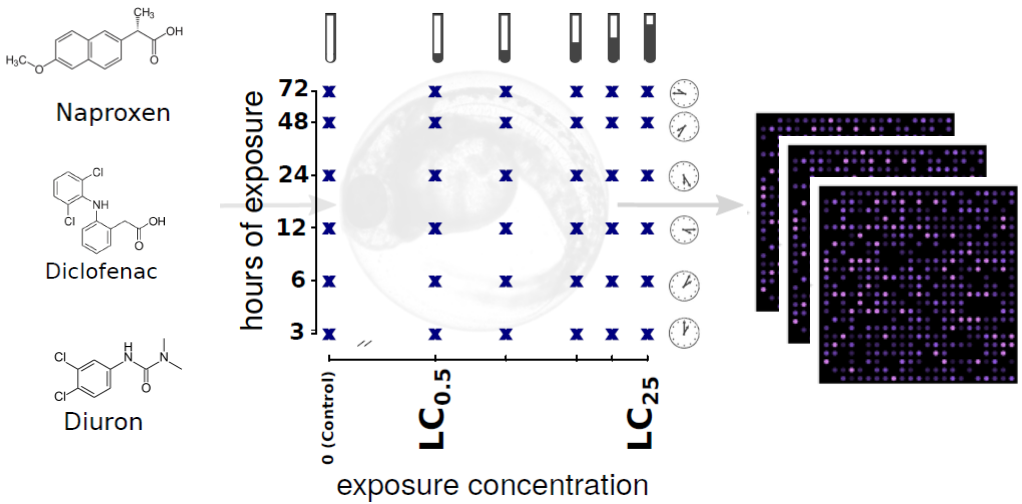
Experiment



Data analysis

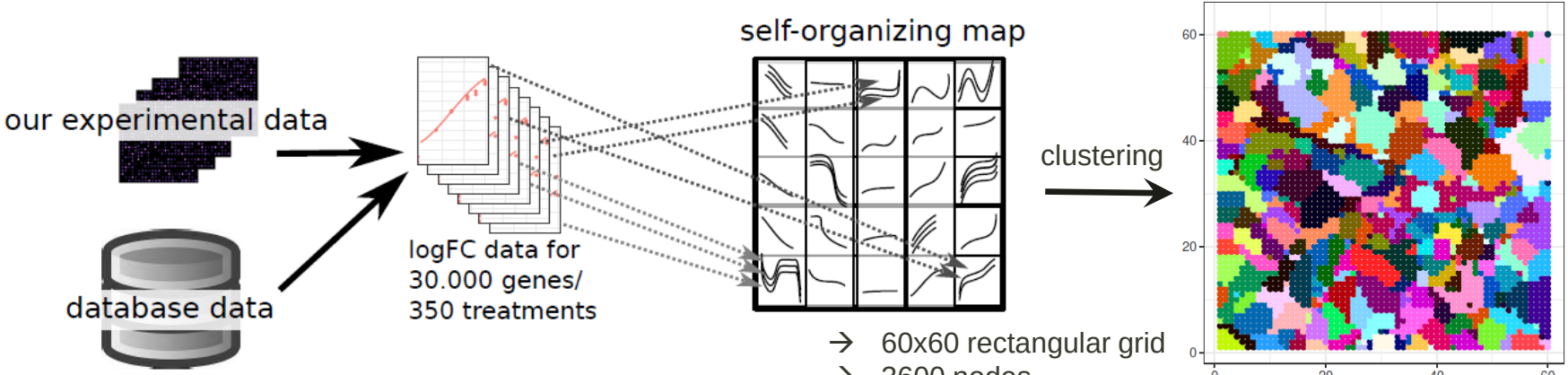
→ *integration* of previous data

→ *aggregation* of fingerprints

→ *modeling* of responses

Schüttler et al., *GigaScience,* 2019

Integration and aggregation of data



→ 60x60 rectangular grid
→ 3600 nodes
→ 118 clusters

Schüttler et al., *GigaScience,* 2019

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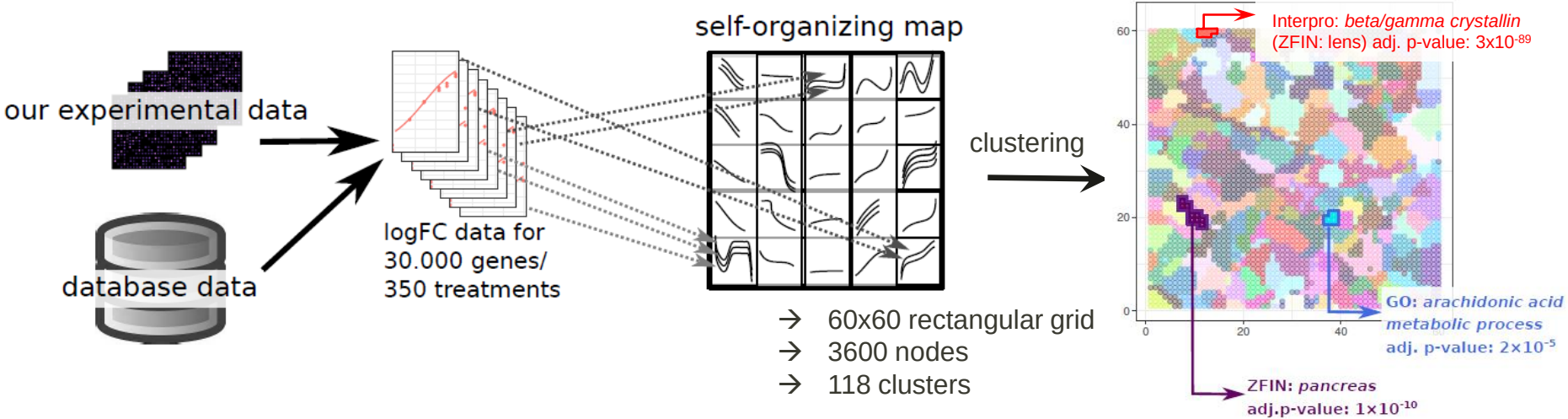

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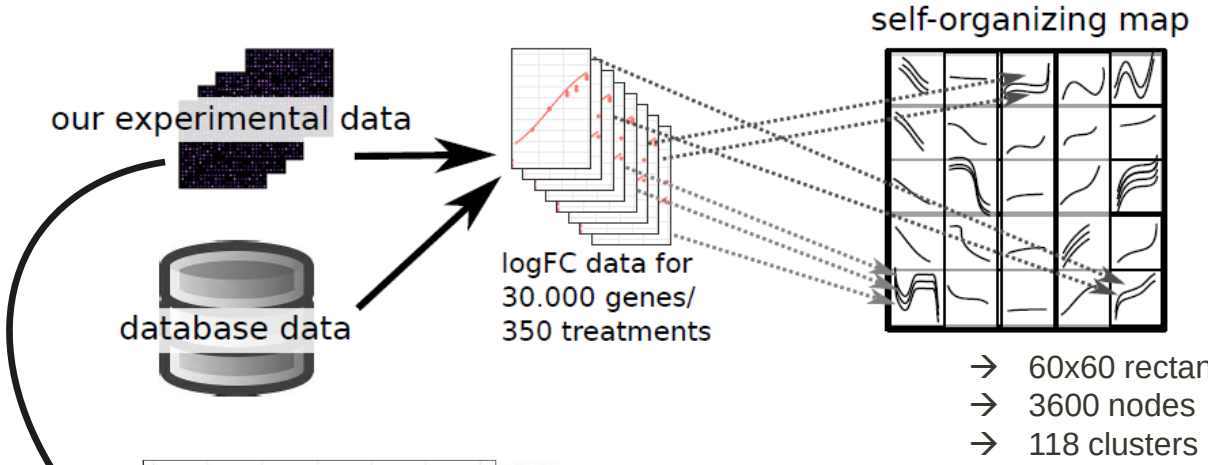

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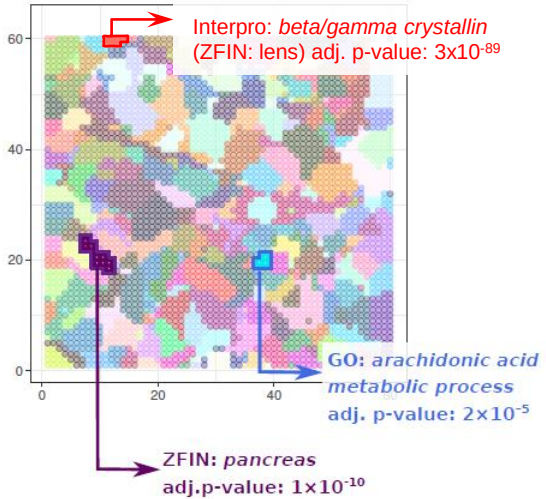
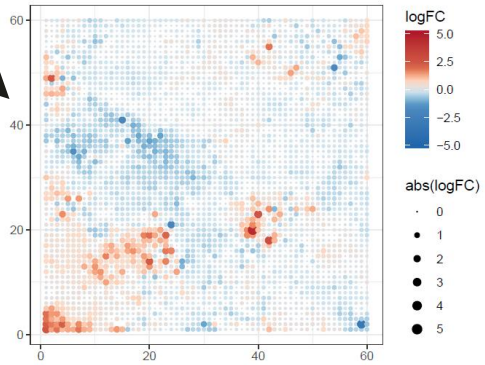
functional enrichment

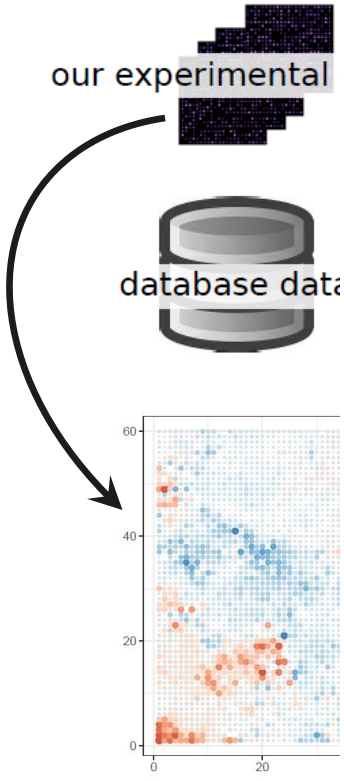




Diclofenac, 48 hours exposure, 7.4 µM

Schüttler et al., *GigaScience,* 2019

Integration and aggregation of data



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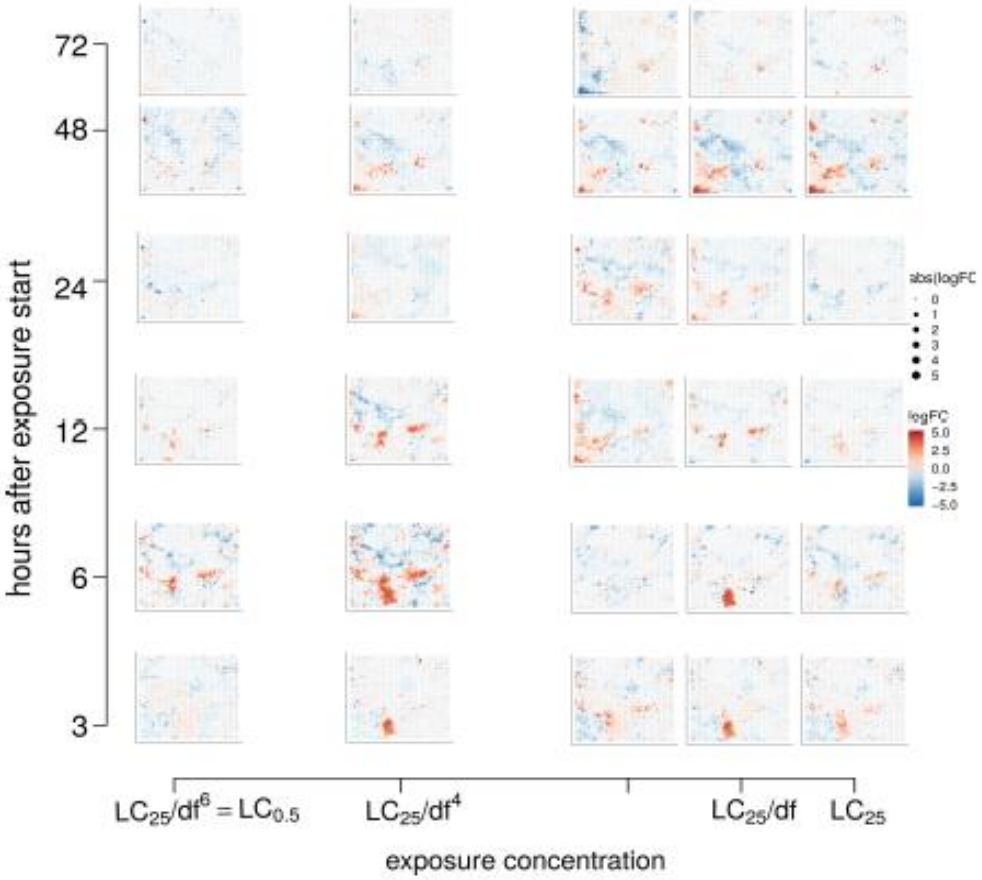

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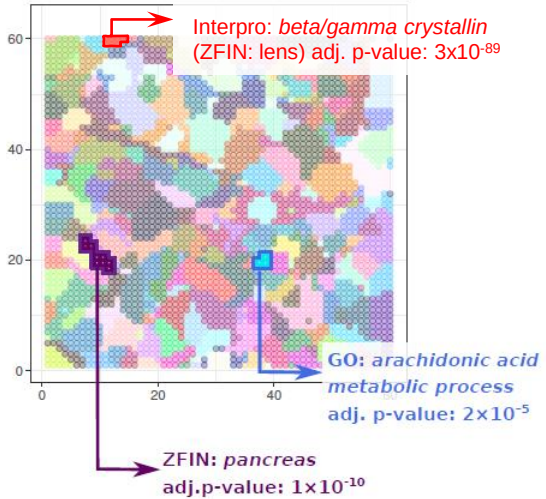

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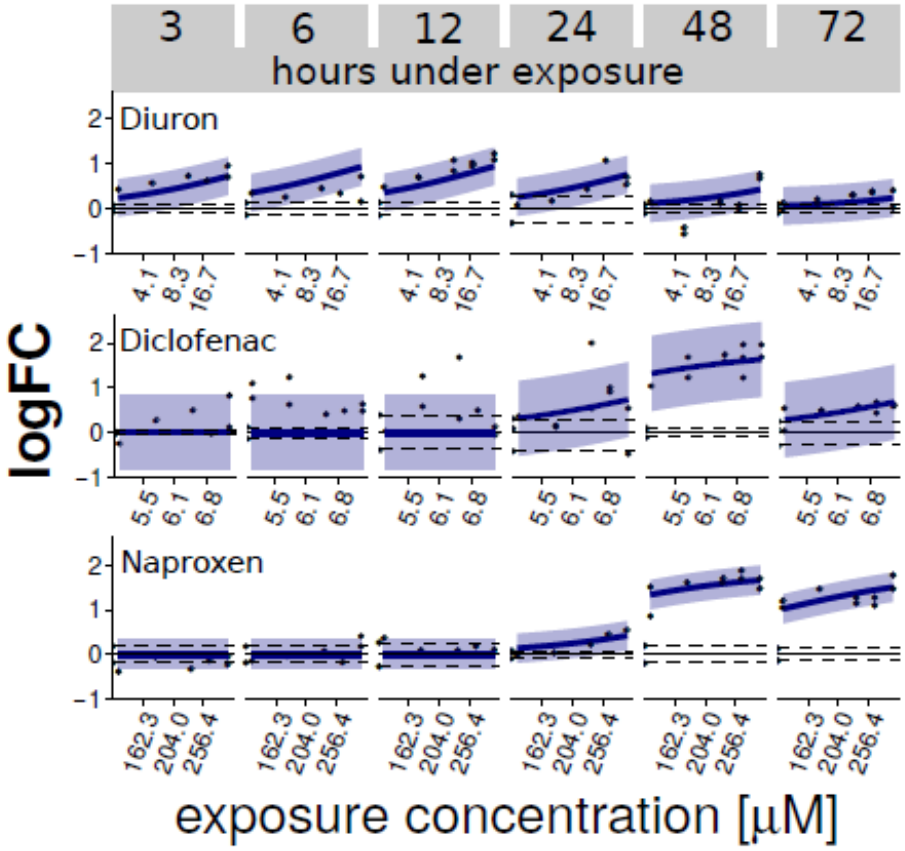
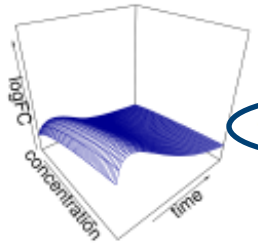
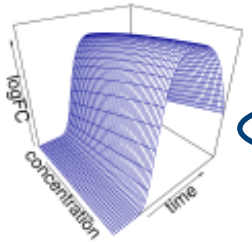
Regression modeling

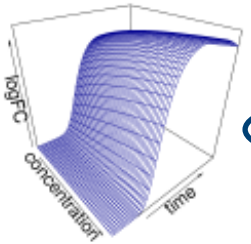




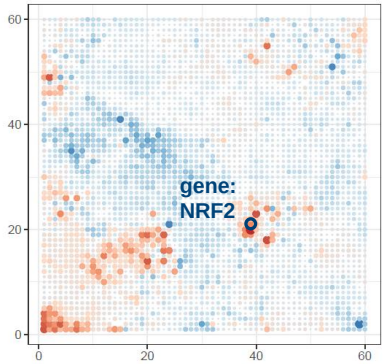
S_{max}: 0.041
S_{dur}: 0.8
t_{max}: 8.8
slope: 0.6
σ: 0.3



S_{max}: 0.283
S_{dur}: 0.42
t_{max}: 41.3
slope: 3.2
σ: 0.5



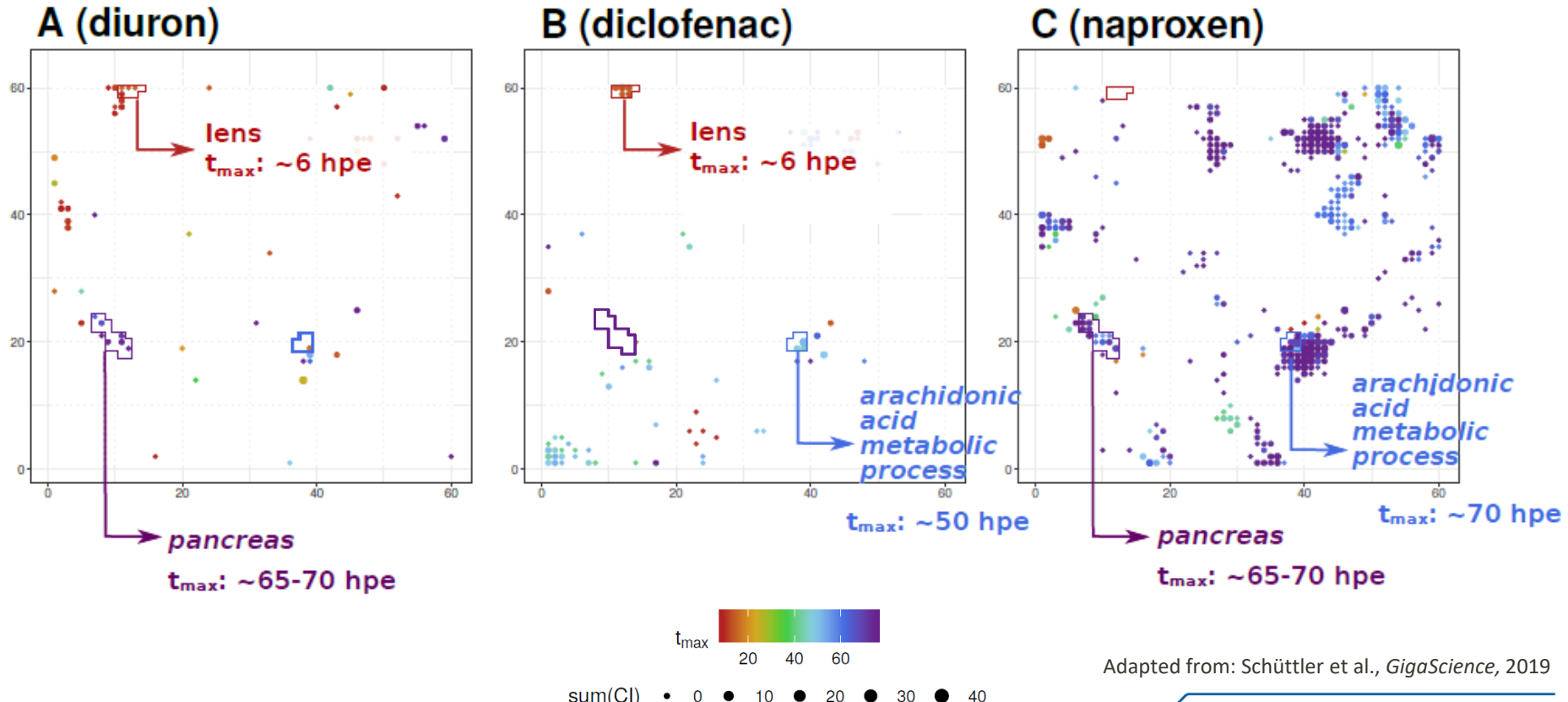
S_{max}: 0.014
S_{dur}: 0.35
t_{max}: 50.6
slope: 1.6
σ: 0.2



Mapping model parameters

Herbicide (PSII Inhibitor)

Pharmaceuticals (Cox-inhibitors)



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

Mixture effects on the transcriptome

- Recovery of single substance effects?
- Occurence 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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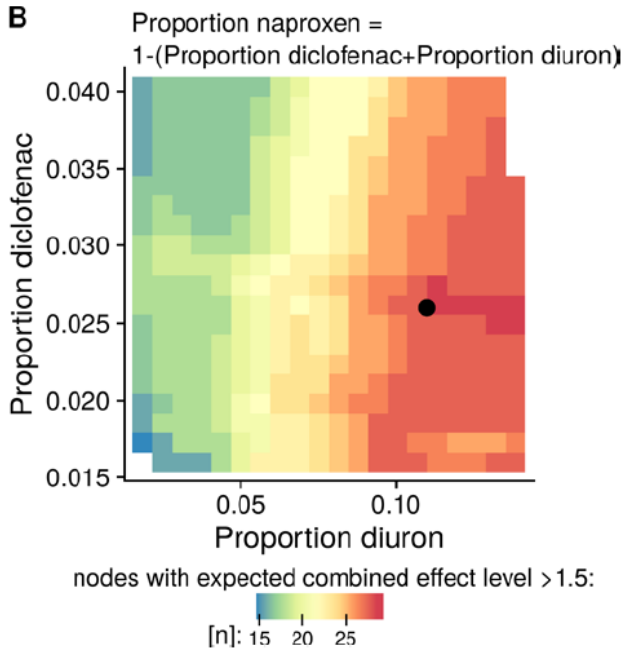

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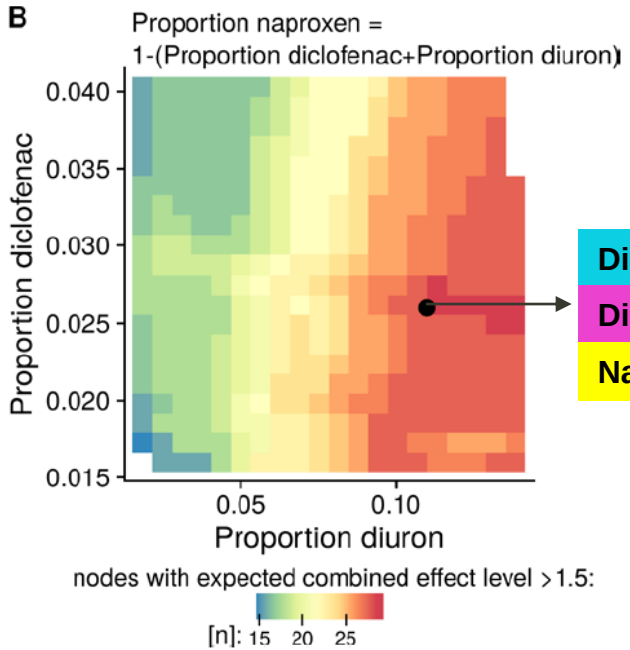
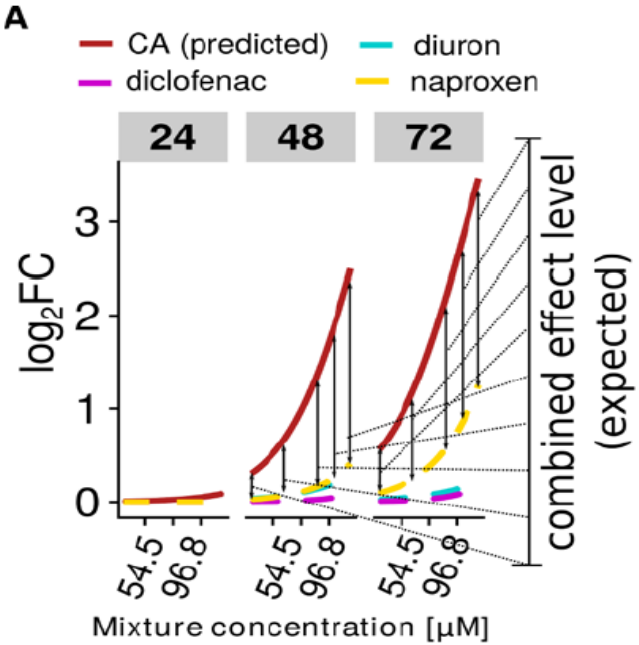
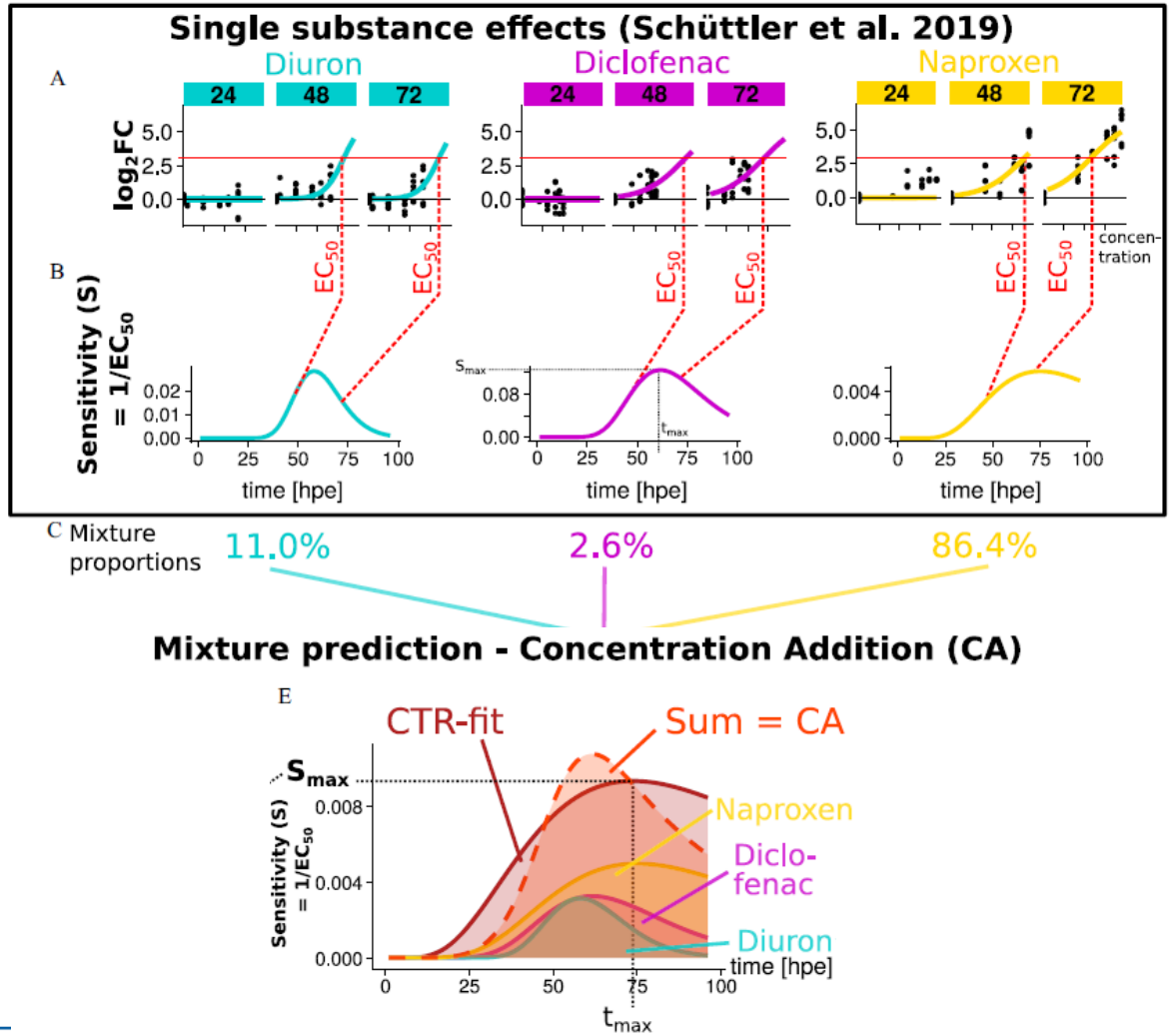


Diuron:	**11%**
Diclofenac:	**2.6%**
Naproxen:	**86.4%**



Mixture effect prediction



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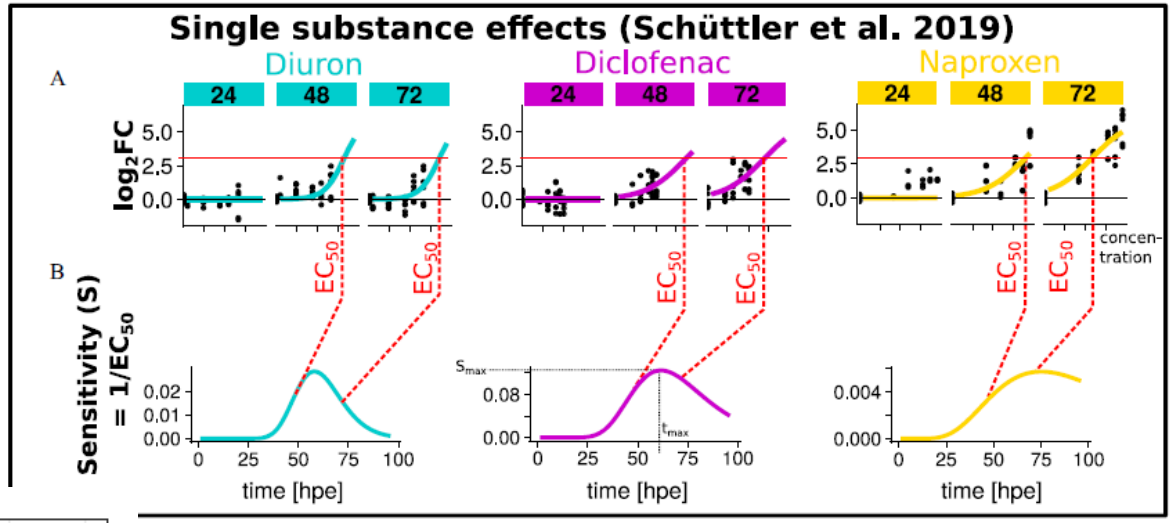
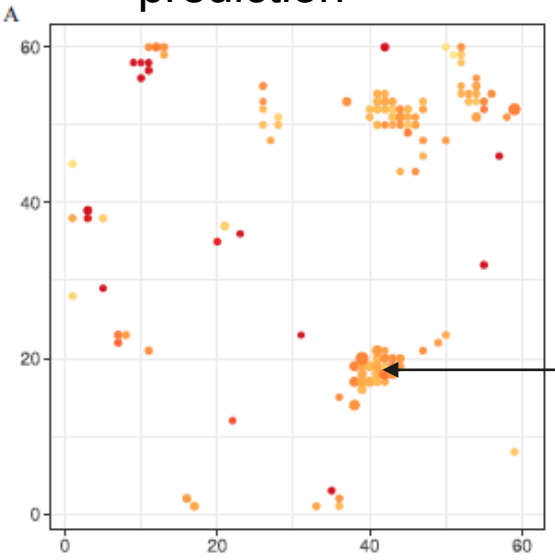
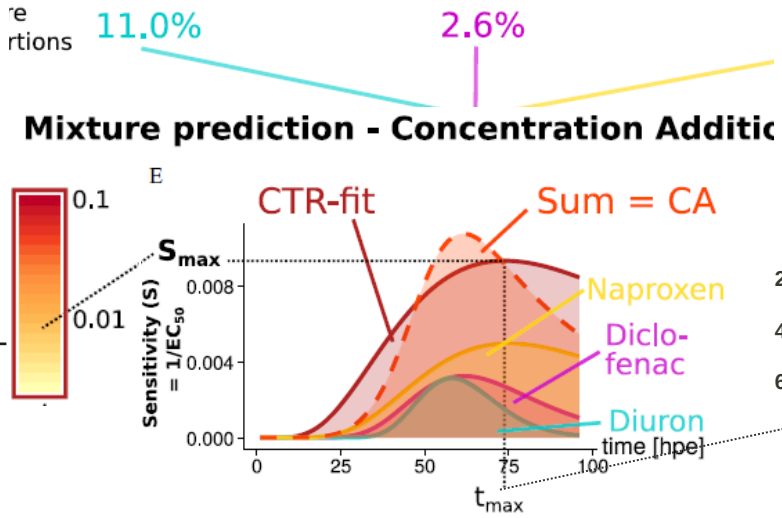
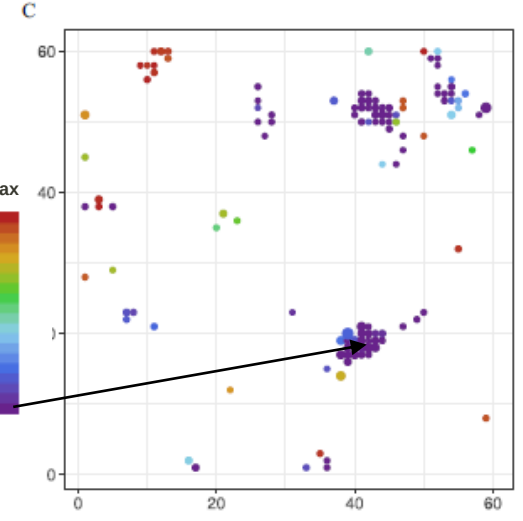


S_{max} map – prediction





t_{max} map – prediction



Mixture effect prediction

Predicted
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(with CA concept)
= **122 nodes**

Schüttler et al., *Environmental Health Perspectives,* 2021

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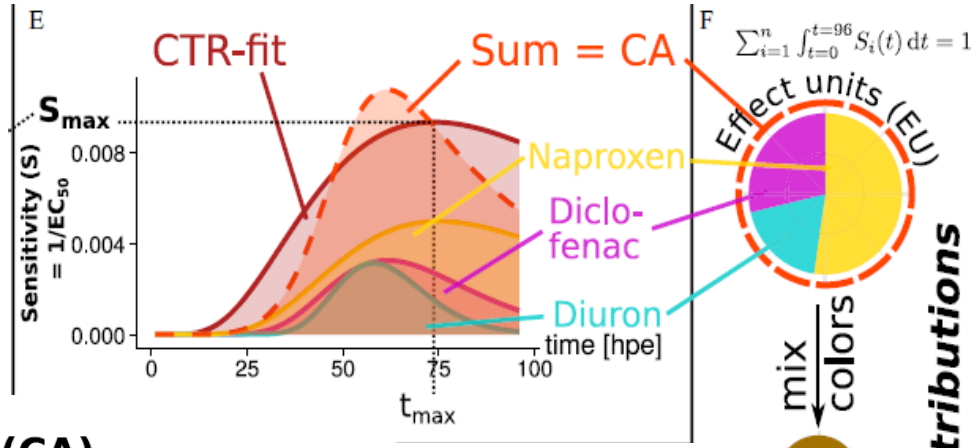
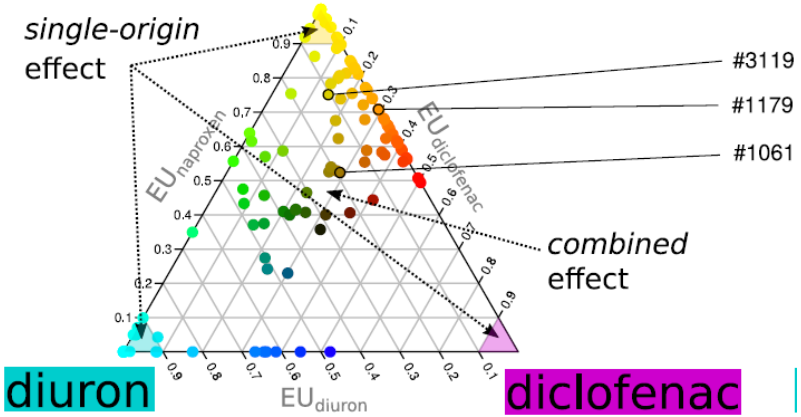
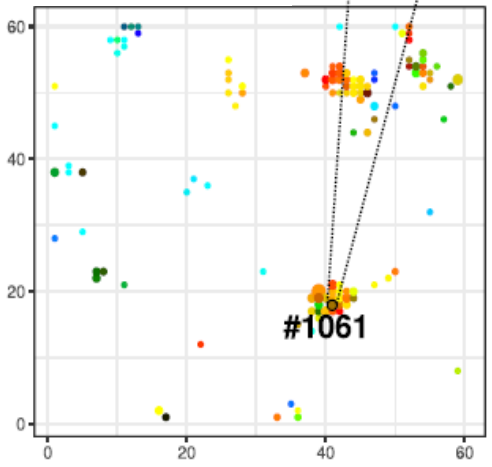


Prediction (CA)



EU map - prediction



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Mixture effect measurement

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

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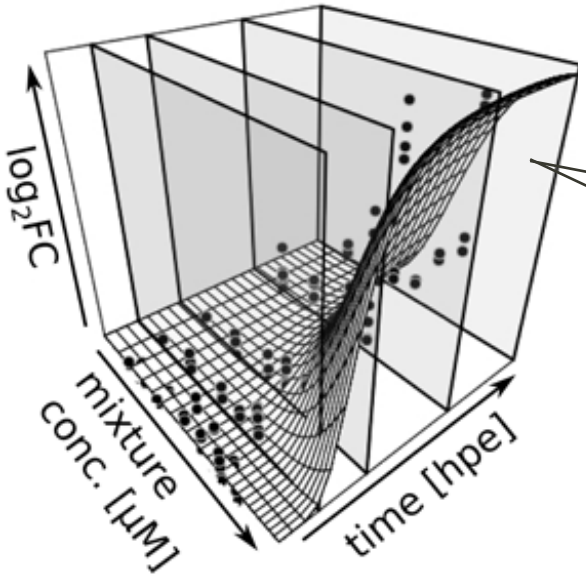
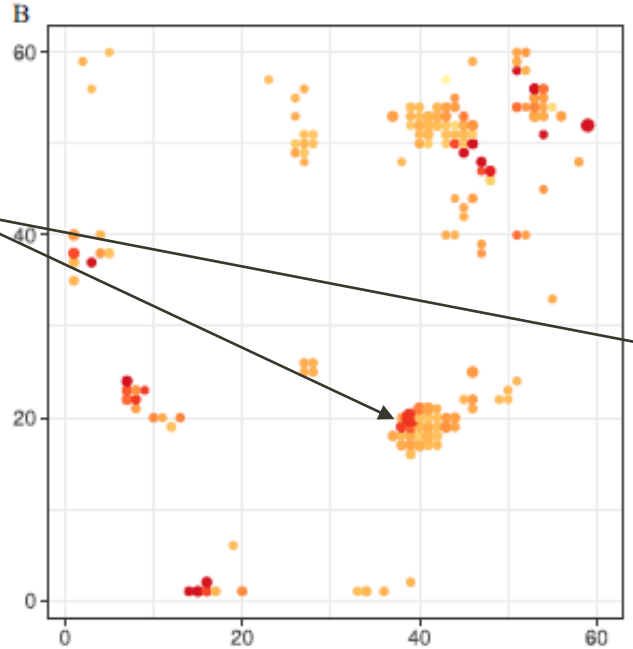
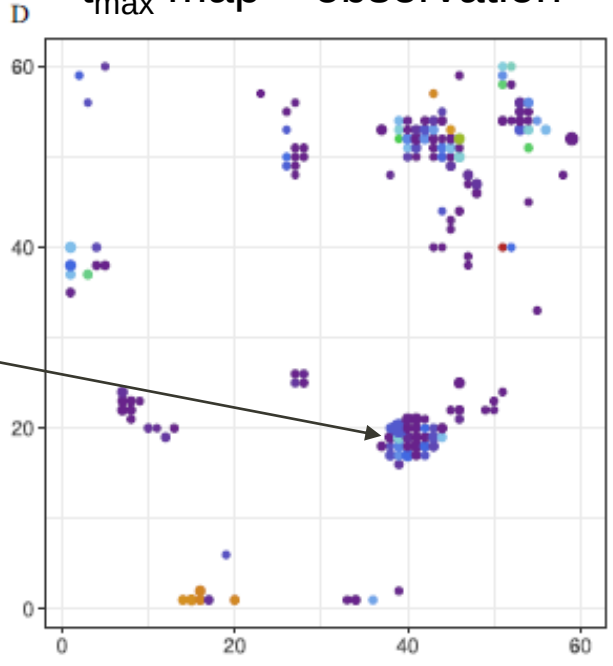


S_{max} map – observation



t_{max} map – observation



Prediction vs Observation

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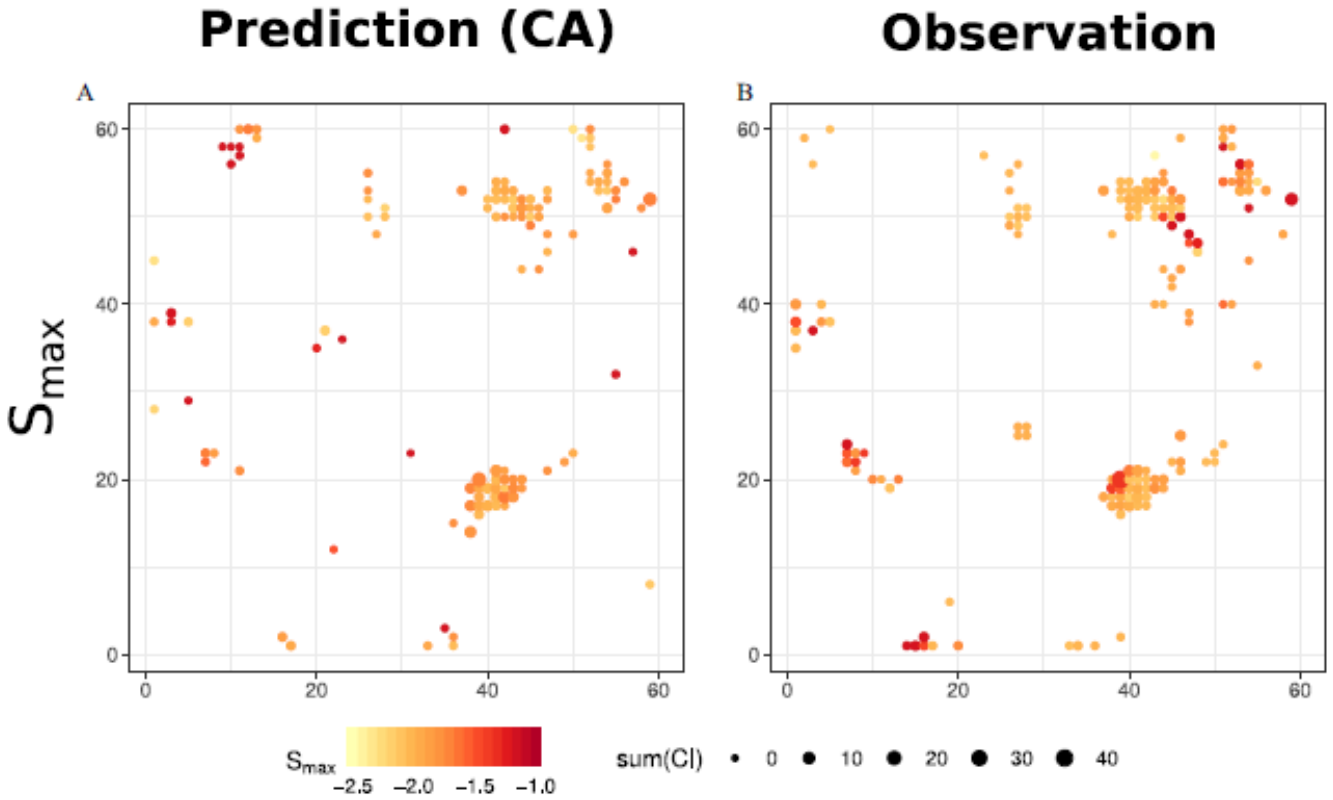

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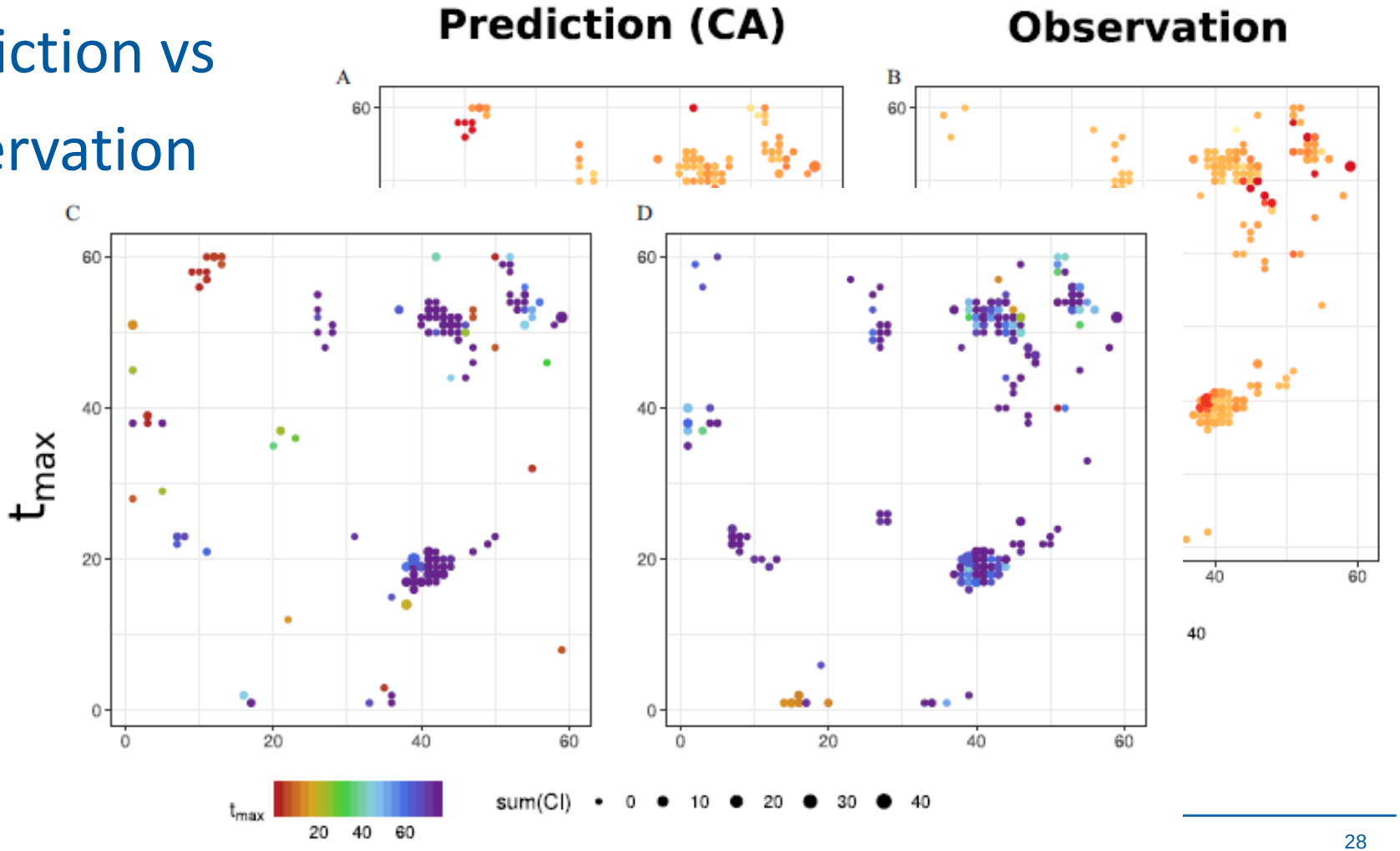
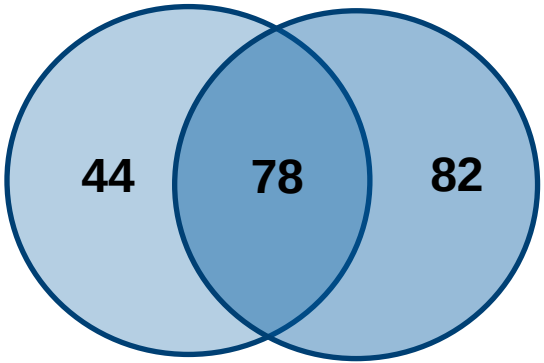


Prediction vs Observation

Significantly affected



Predicted to be affected: **122 nodes**

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Total: **204 node**

Prediction vs Observation

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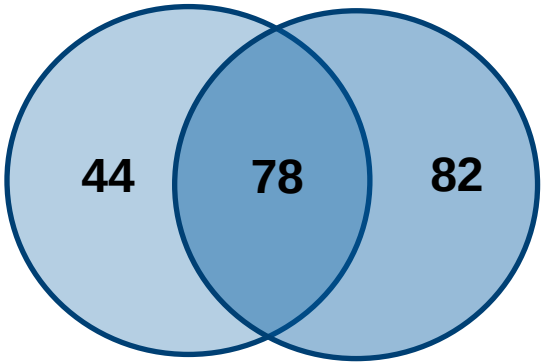

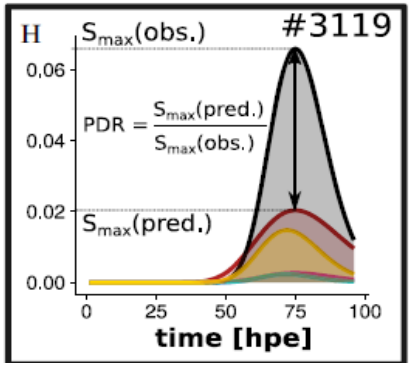

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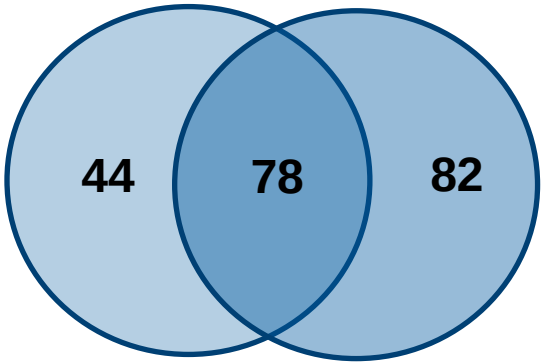

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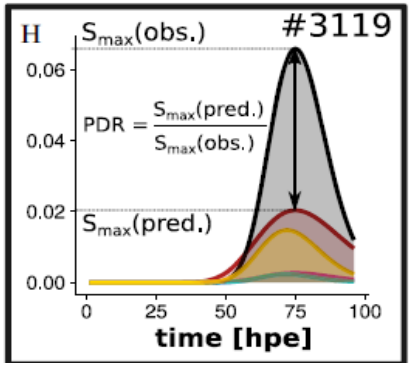
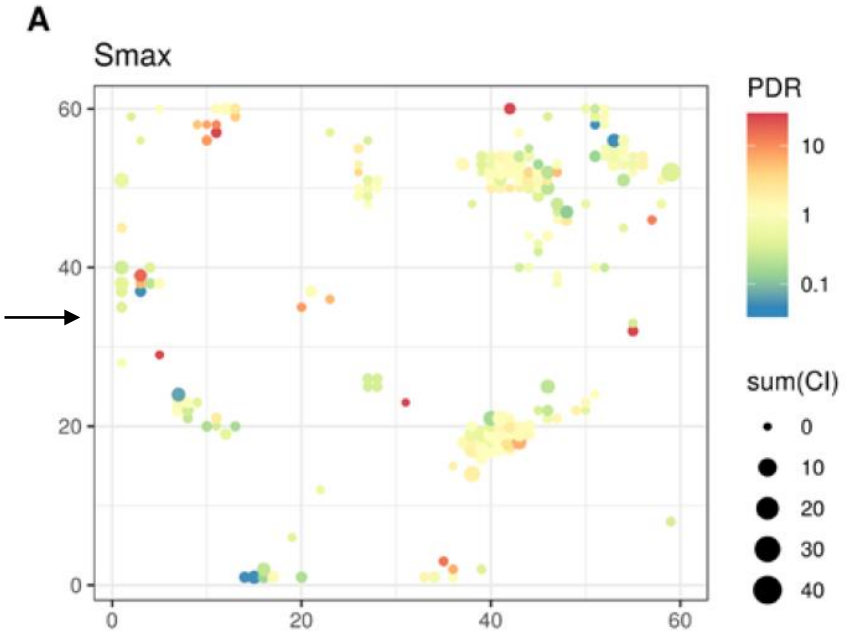
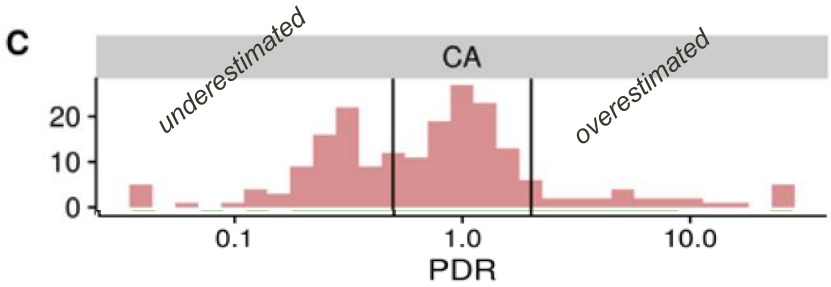


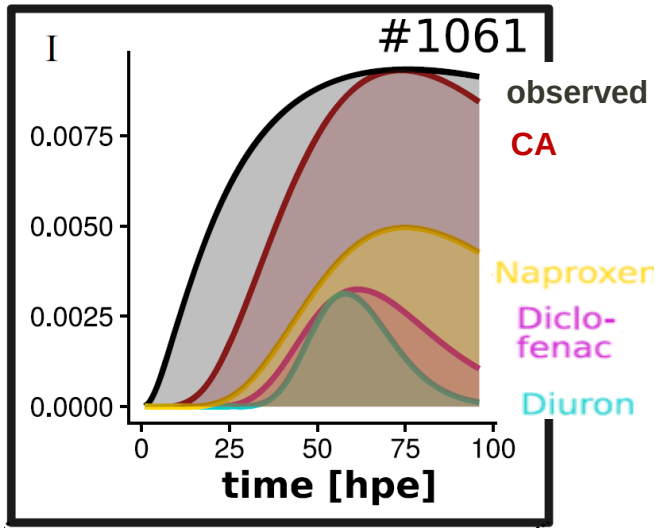




Deviations from Predictions

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

Combined effect



GTP binding proteins

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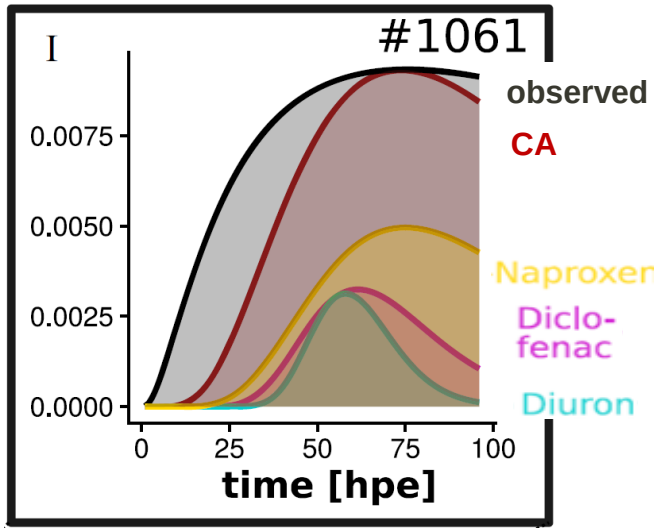

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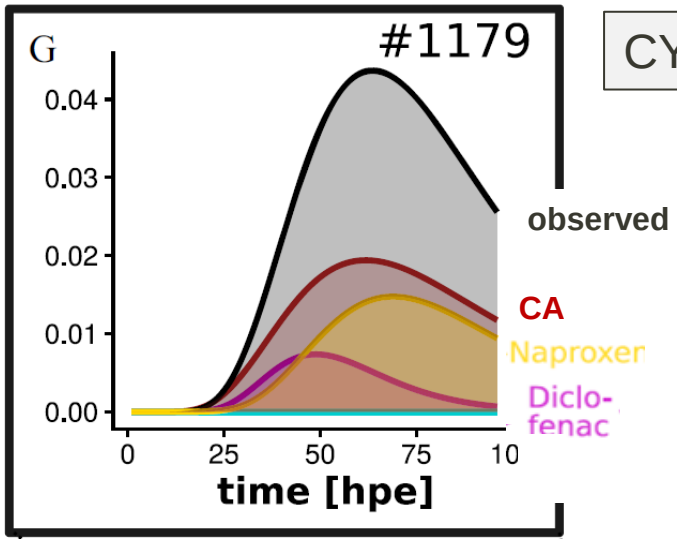
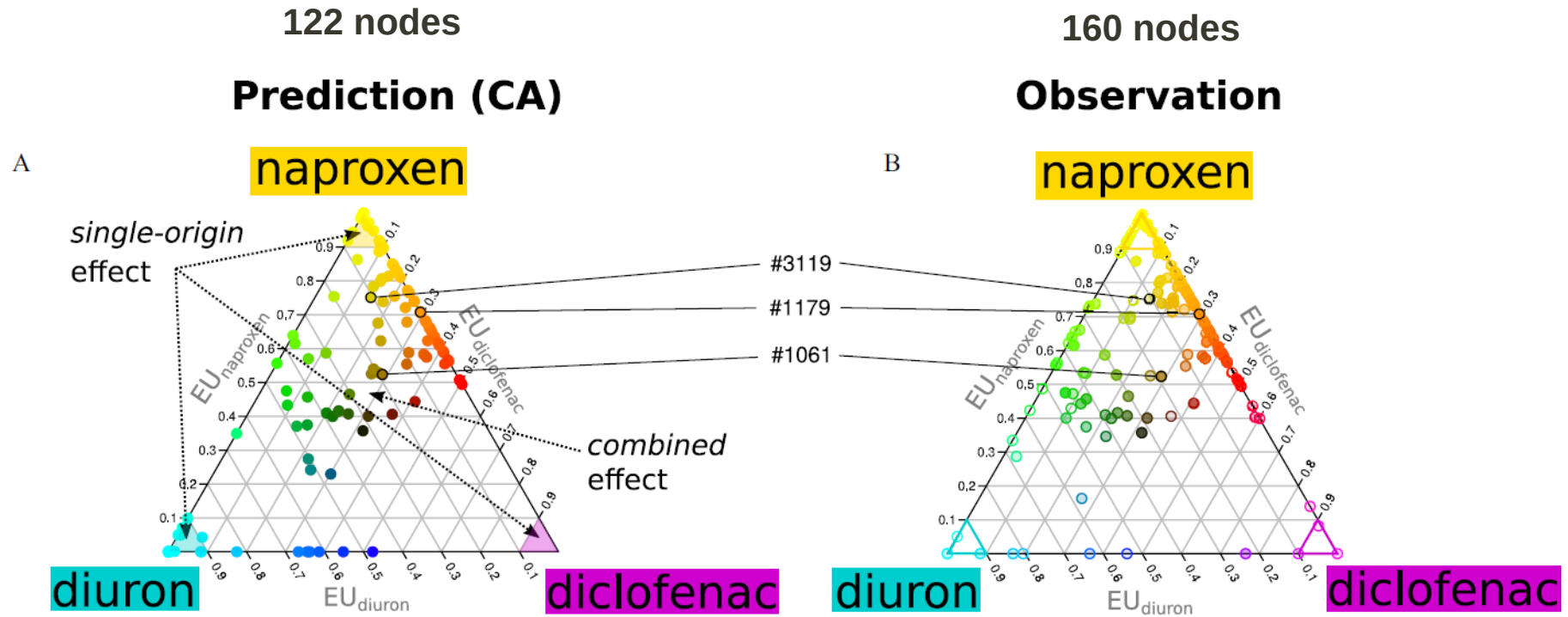
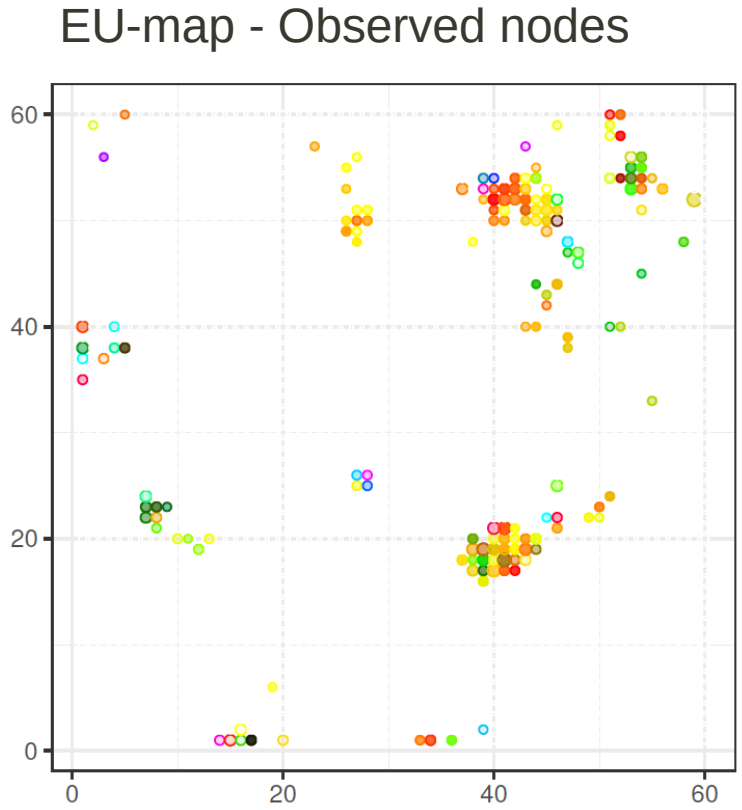


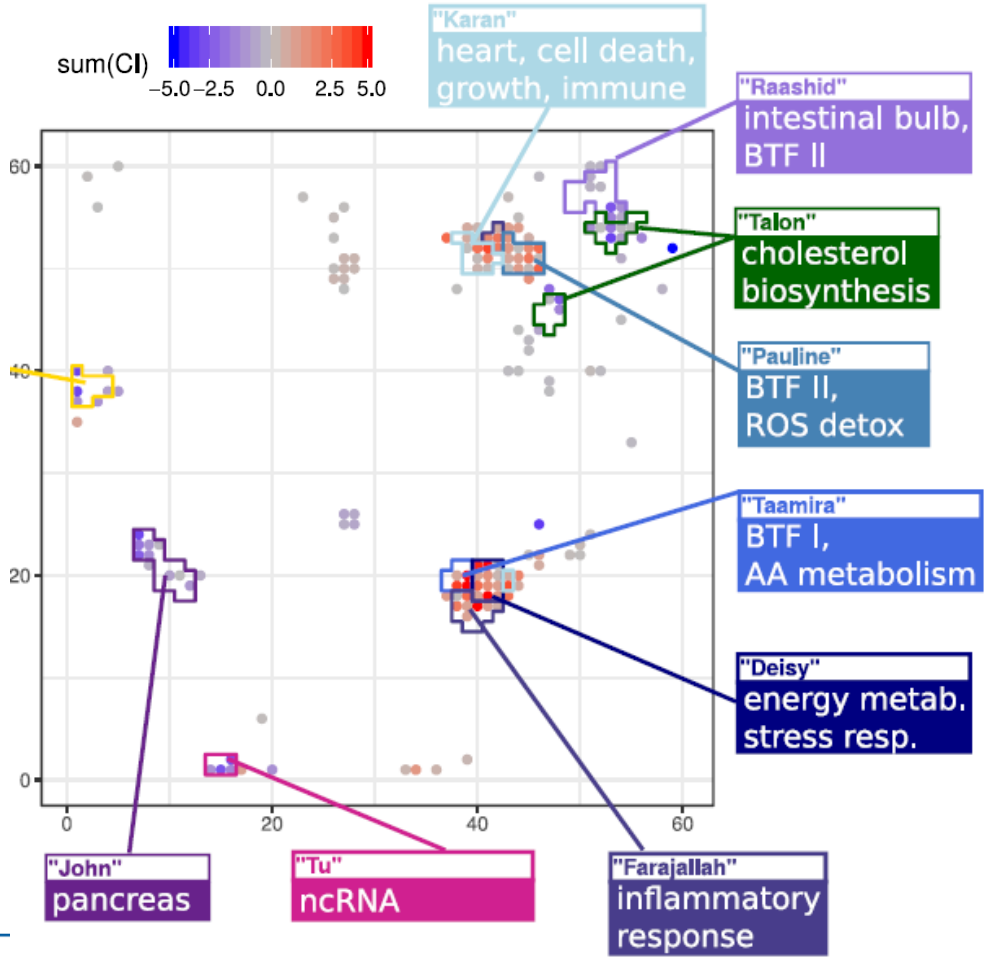
CYP2K18

Prediction vs Observation - Component contributions



Joint action of similar and dissimilar compounds





Summary | Conclusion

- Recovery of single substance effects? **YES**
- Occurrence of combined effects on the transcriptome? **YES**

  → Combined effects observed also for anticipated dissimilar acting compounds

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→ **Proof of concept:** molecular effects are not random and can be predicted for mixtures comprehensively based on individual component knowledge

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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*