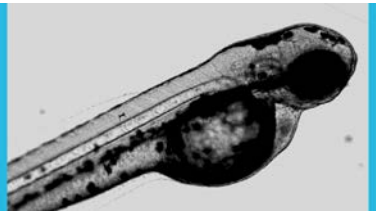
SUBSTANCE-SPECIFIC FINGERPRINTS OF THYROID DISRUPTION IN ZEBRAFISH EMBRYOS



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Fraunhofer IME

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Background

Endocrine disrupting chemicals (EDCs)

EDCs are chemicals that interfere with the endocrine system & trigger an adverse development, reproductive and immune effects to exposed organism or its descendant population

OECD TGs

- Estrogen
- Androgen
- Thyroid hormones
- Steroidogenesis

in vertebrates

functions
- normal physiology
- development
- growth
- energy metabolism

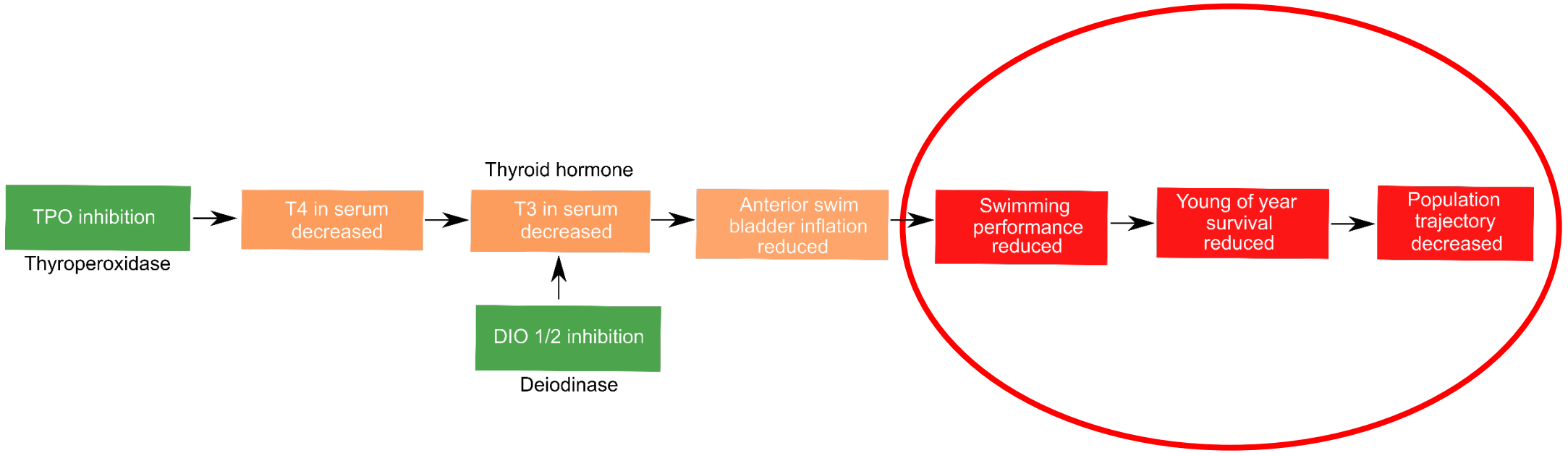
Many organic compounds in the environment can interfere with and disrupt the thyroid system

Spaan et al., 2019. https://doi.org/10.1016/j.scitotenv.2018.11.071

Background

Exposure → **Effect**

- Fish studies - an adverse outcome pathway (AOP) was recently proposed linking thyroid disruption to decreased swim bladder inflation and impaired swimming performance in fish

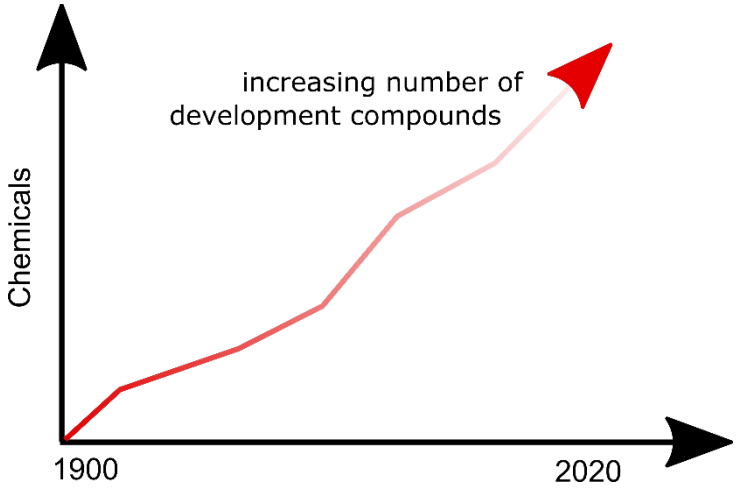


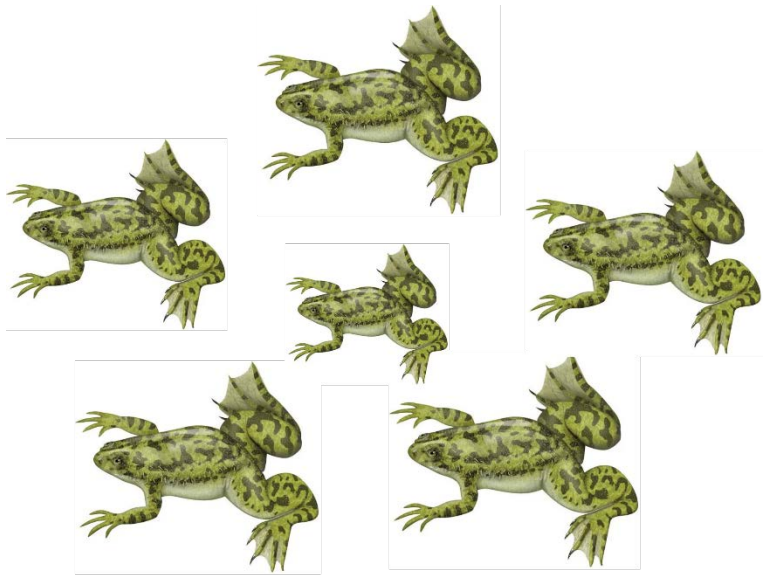
AOP-Wiki Website; https://www.aopwiki.org

Background

Current assessment of endocrine substances targeting thyroid hormone-related physiological processes, focuses on:

- Xenopus Embryo Thyroid Signaling Assay (XETA) (OECD, 2019)
- Amphibian Metamorphosis Assay (AMA) (OECD, 2009)
- Larval Amphibian Growth & Development Assay (LAGDA) (OECD, 2015)





Amphibians/Chemicals

The required number of amphibians needed for these tests strongly contrasts the 3R principle of:

- reduction
- replacement and
- refinement of animal experiments

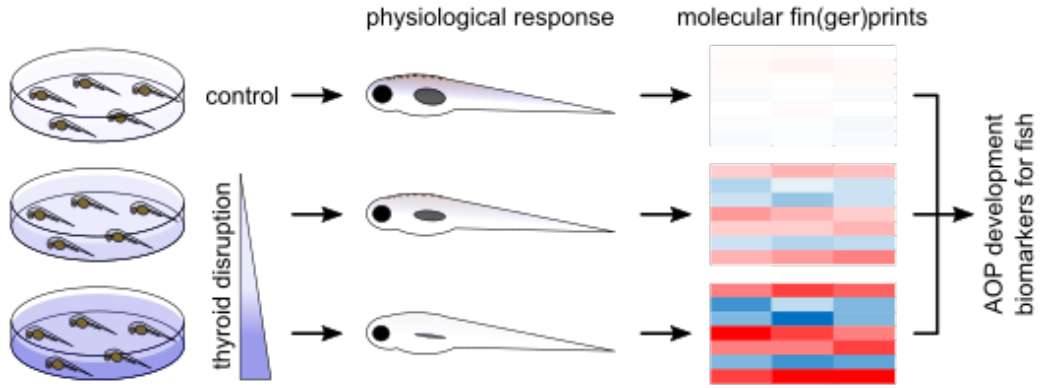
Test Strategy

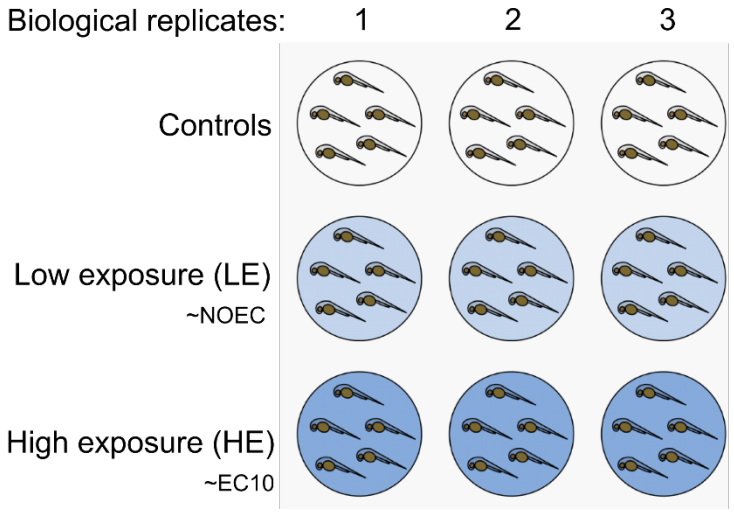
Test strategy

Objective

- To demonstrate the sensitivity of -omics based approaches in assessing thyroid disruption in zebrafish embryo

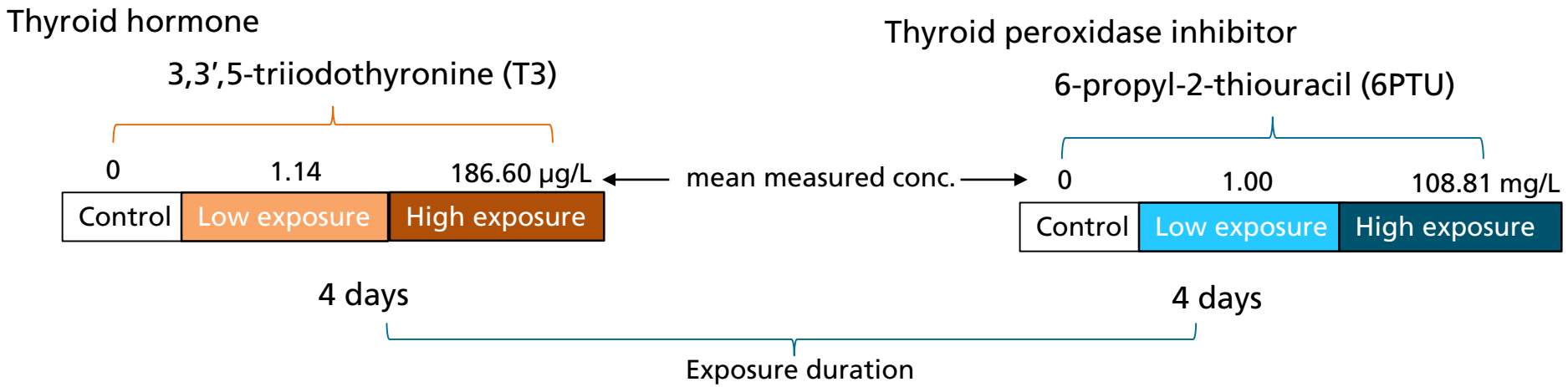
Experimental design





- Semi-static incubation for 96 hr
- 3 biological replicates
- 15 embryos per well

Test substances

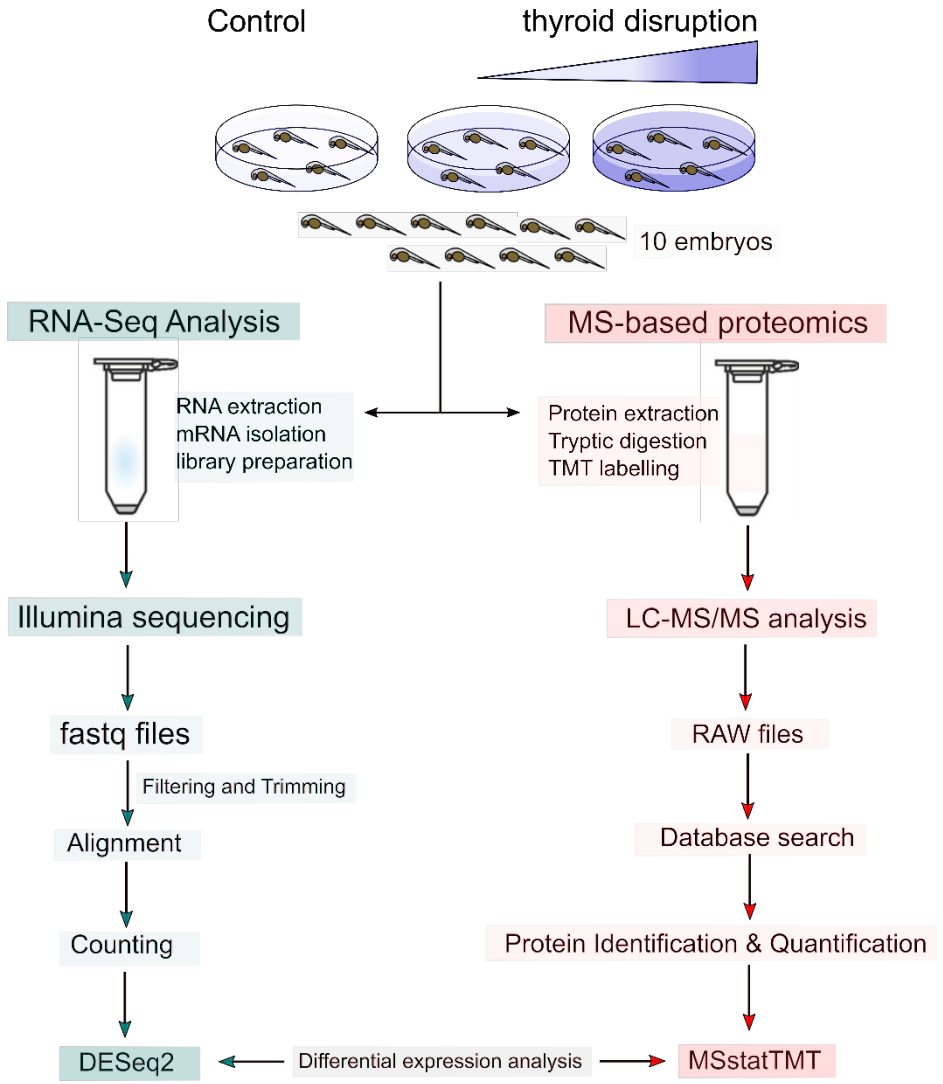


Water analysis

T3 (µg/L)		6-PTU (mg/L)	
nominal	Ø measured (n = 3x3)	nominal	Ø measured (n = 3x3)
0	0	0	0
1.0	0.23	0.1	0.06
3.3	1.14	1.0	1.00
10.0	2.35	10.0	8.39
330.0	186.60	100.0	108.81

→ Transcriptome & proteome expression profile

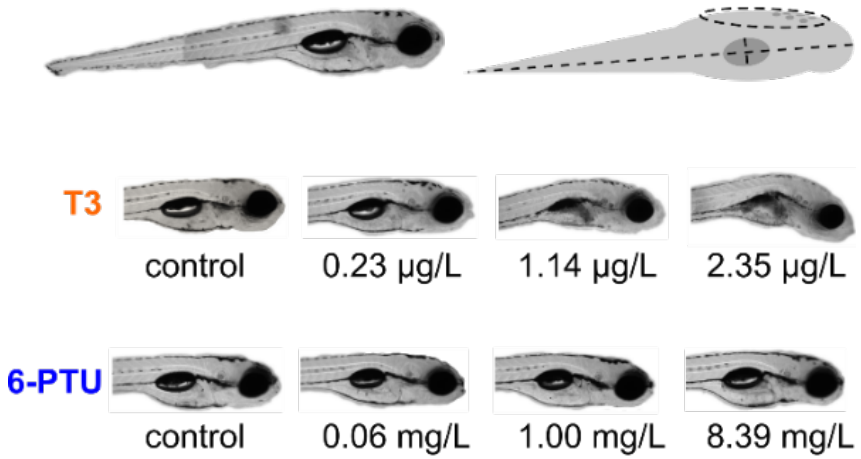
Transcriptomics and Proteomics workflow

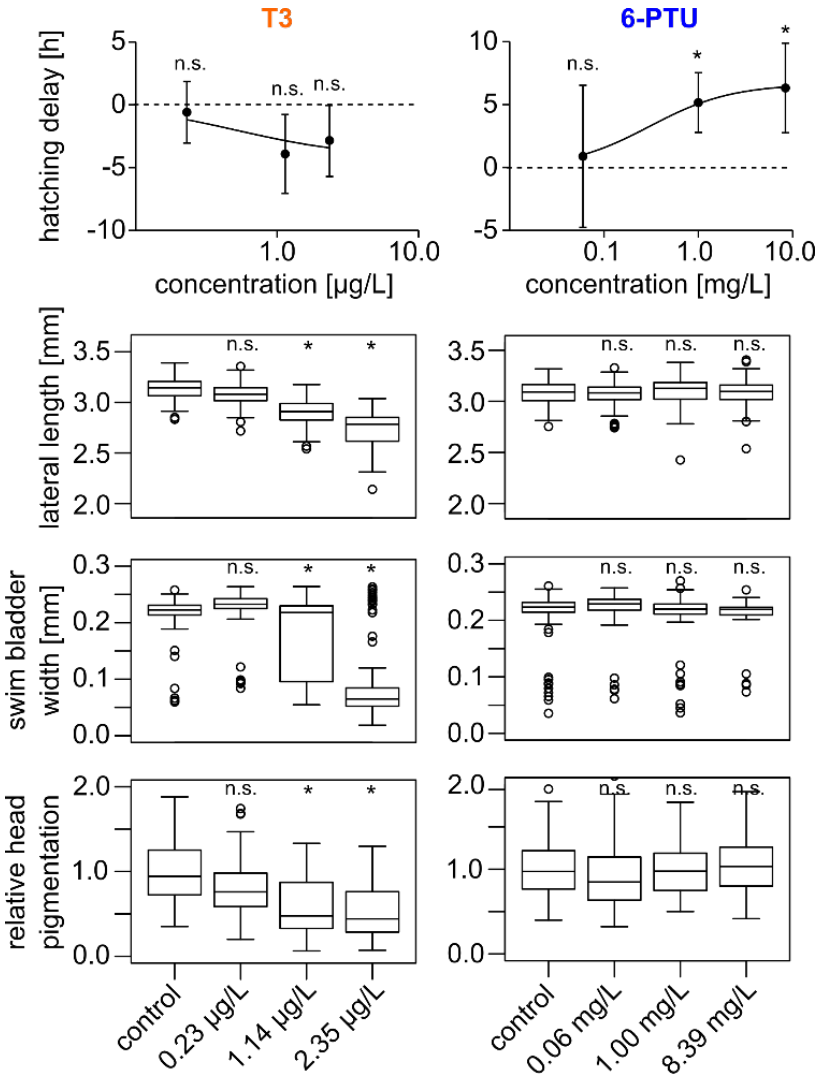


Result

Physiological responses

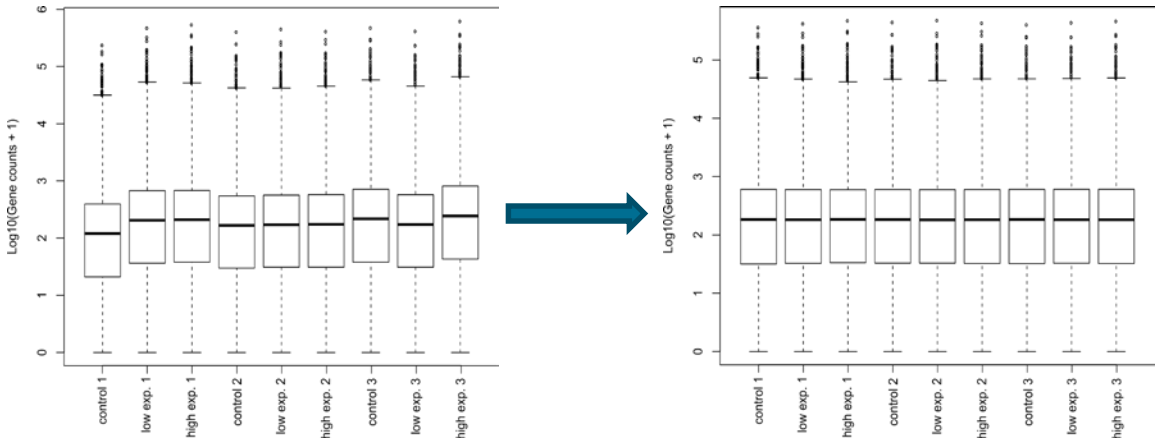
Physiological effects



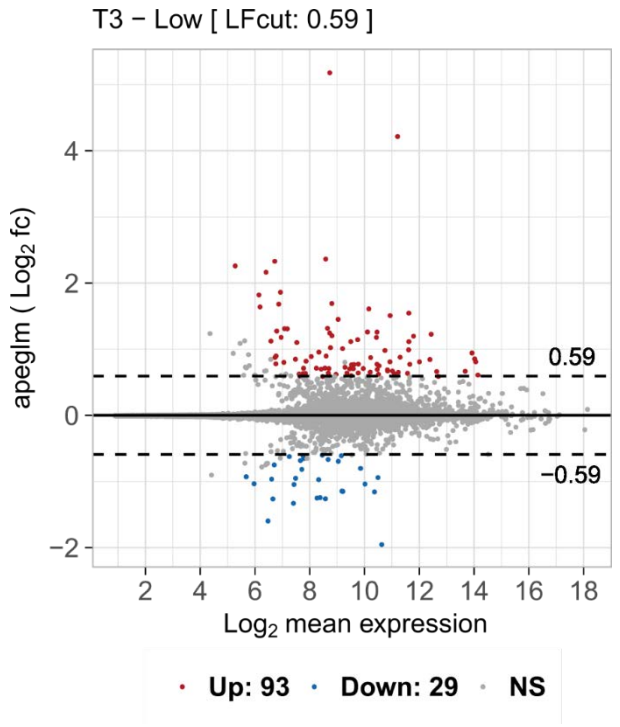


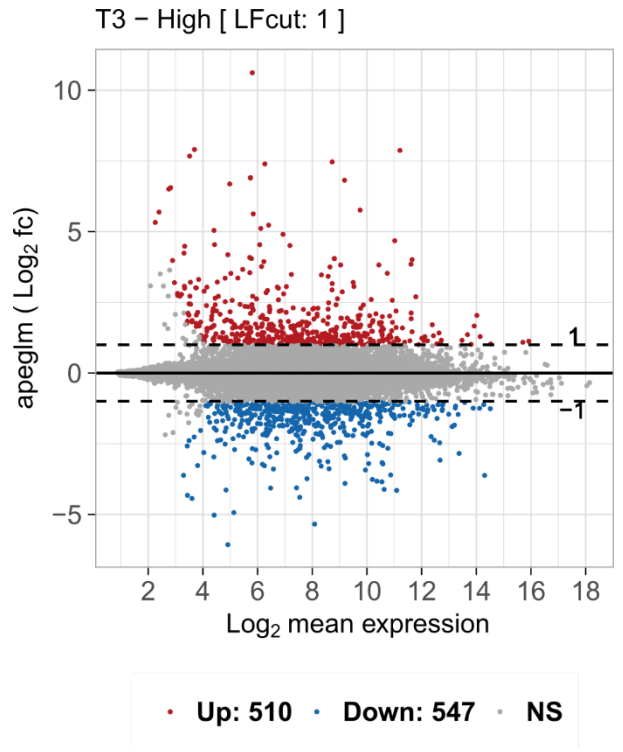
Transcriptomics & Proteomics Result

Normalization - Transcriptomics data

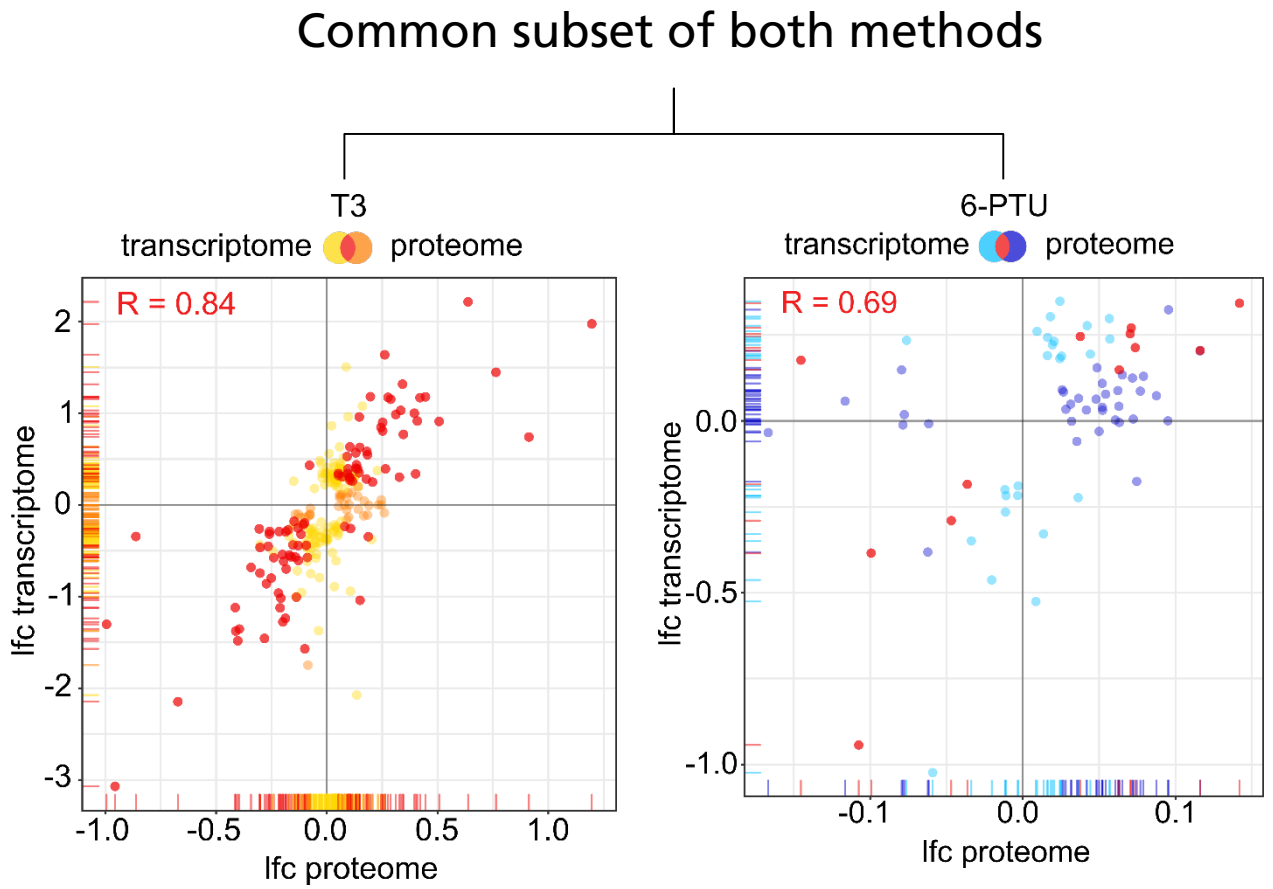


Differentially Expressed Genes (DEGs)





Overlap of transcriptome and proteome data

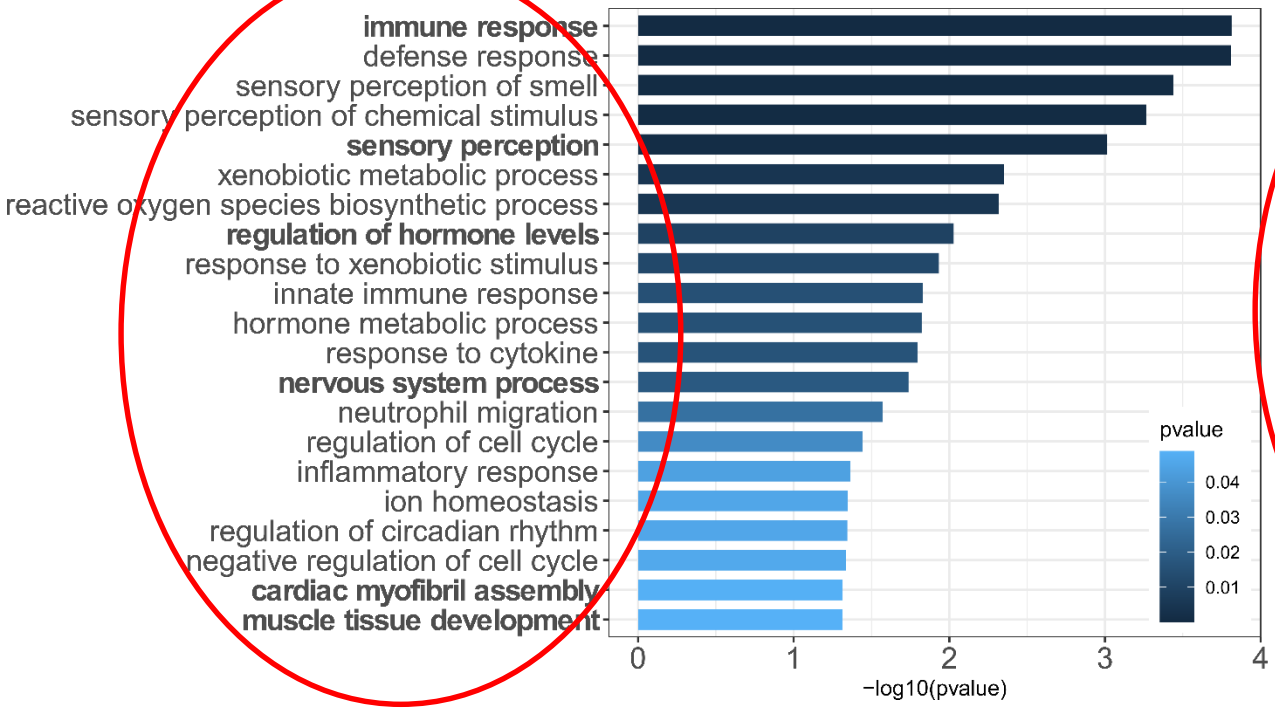


Gene Set Enrichment Analysis

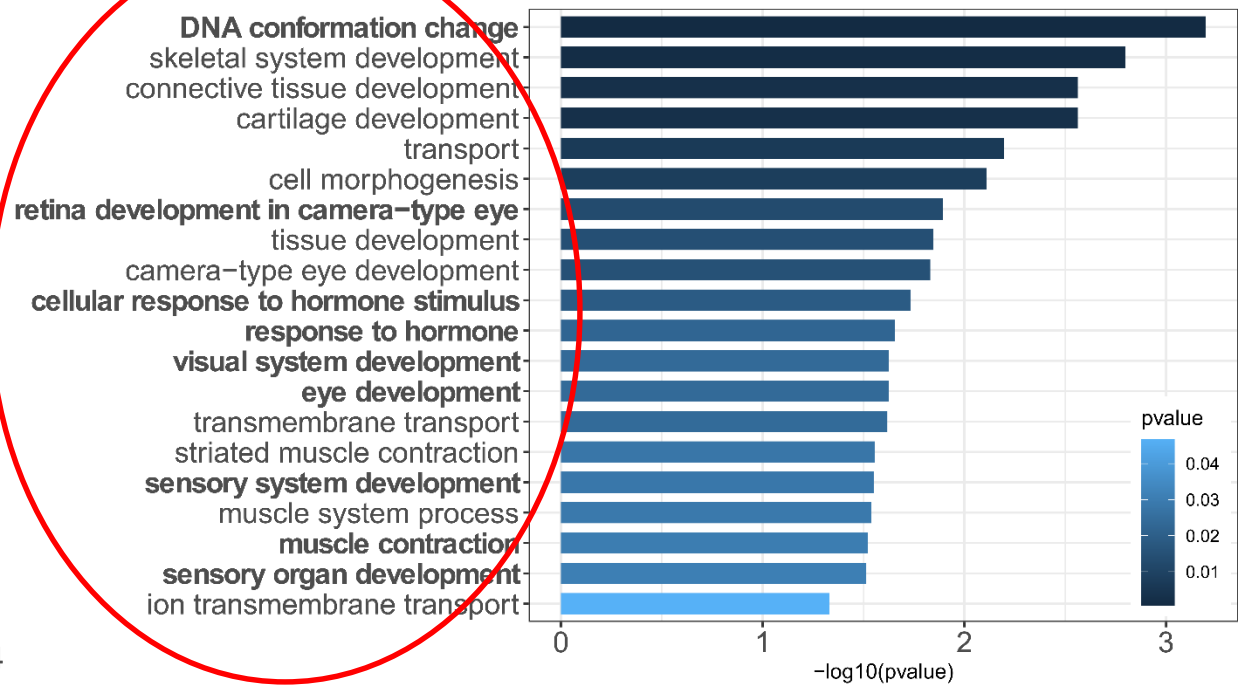
Gene Set Enrichment Analysis

6PTU-exposure

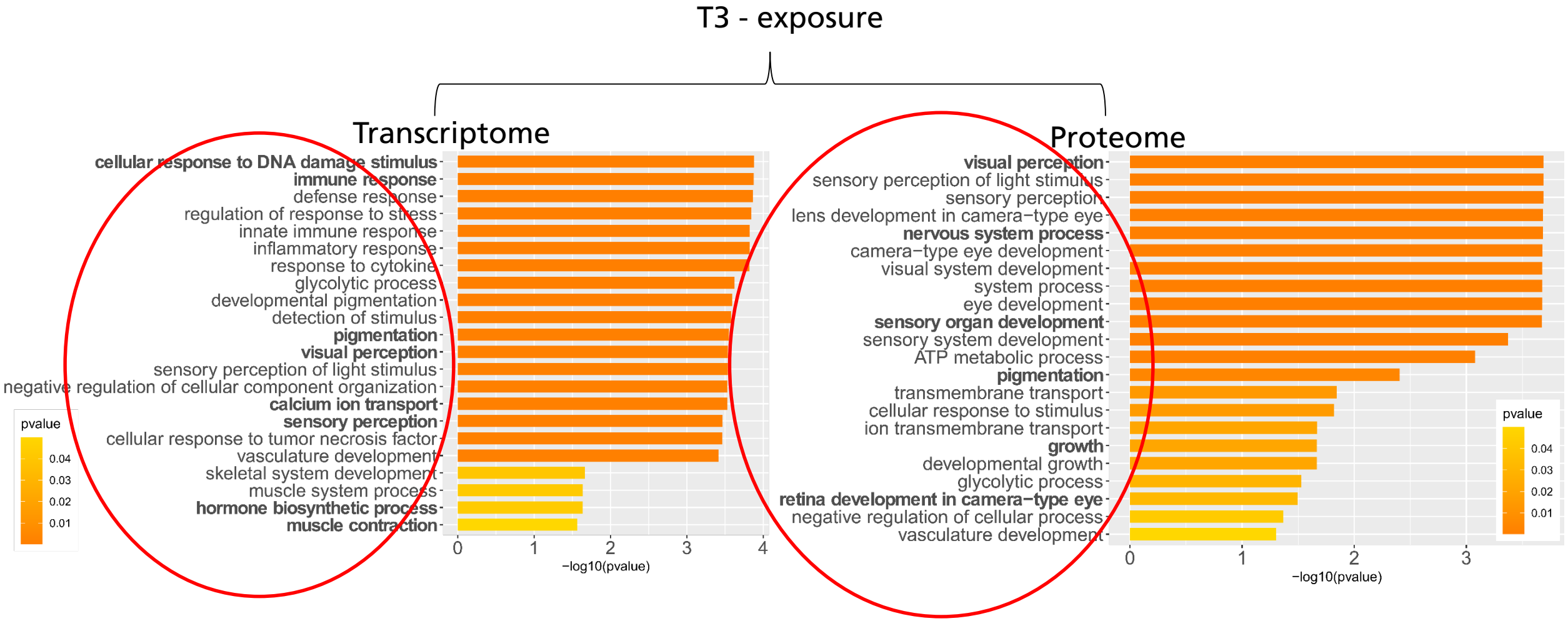
Transcriptome



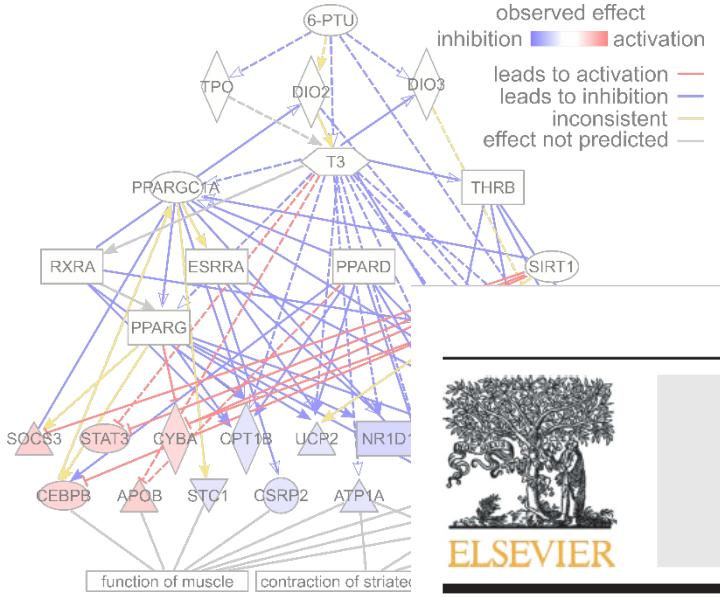
Proteome

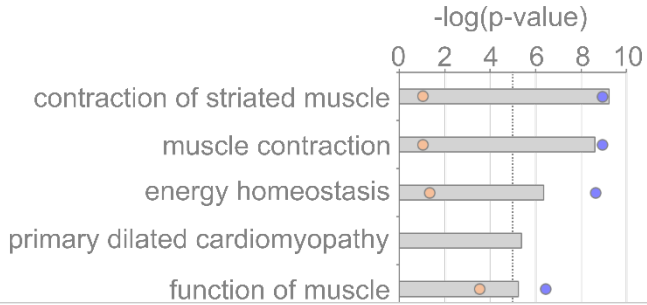


Gene Set Enrichment Analysis



To Conclude





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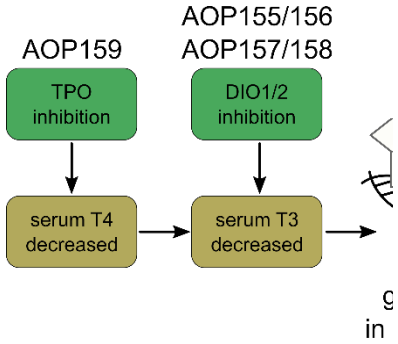
Thyroidal AOP refinement



Toxicogenomic fin(ger)prints for thyroid disruption AOP refinement and biomarker identification in zebrafish embryos

Hannes Reinwald [a,b,1], Azora König [a,1], Steve U. Ayobahan [a], Julia Alvincz [a], Levente Sipos [a], Bernd Göckener [c], Gisela Böhle [d], Orr Shomroni [e], Henner Hollert [b], Gabriela Salinas [e], Christoph Schäfers [d], Elke Eilebrecht [d], Sebastian Eilebrecht [a,*]

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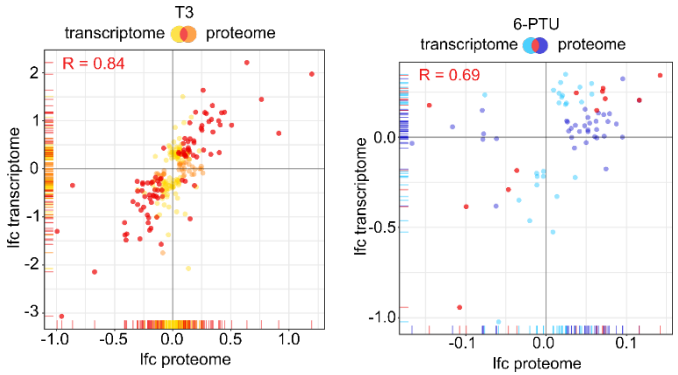
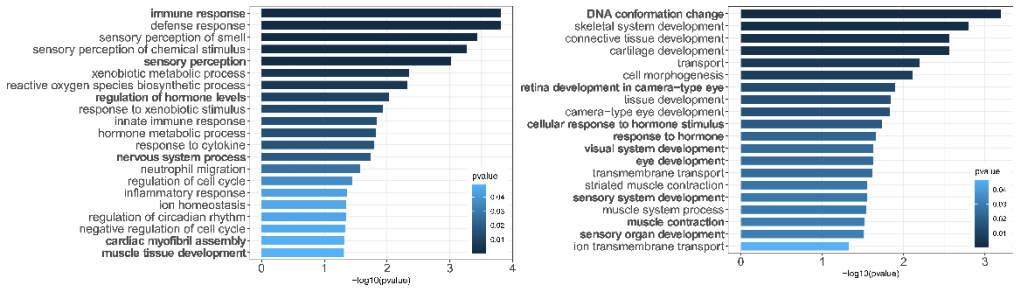
Take home message

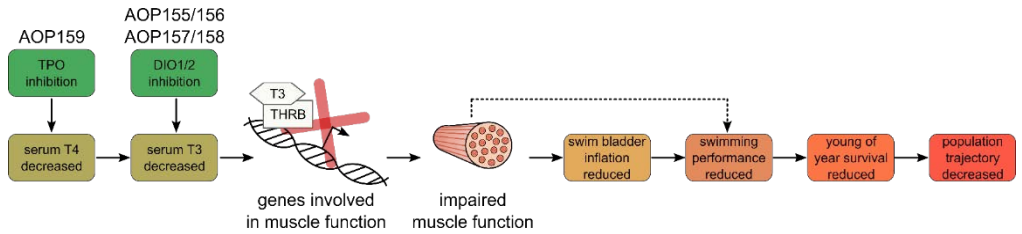
- The **identified transcriptome and proteome fingerprints** can be utilized for understanding thyroid disruption in early zebrafish development.



- Our study demonstrates that **omics-methodology can significantly improve the identification** of endocrine disrupting substances targeting thyroid hormone-related physiological processes.



- Overall, **our findings support the AOP development** for thyroidal ED assessment



Thank you for your attention

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- Julia Alvincz
- Fabian Essfeld (MSc.)
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Reference

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