

Mass spectrometry imaging of the developing amphibian brain: Discovering molecular markers of neurodevelopment Rikke Poulsen^{1,2,3}, Emma M. Field¹, Angela M. Jackson², Haley Kuecks-Winger¹, David Goodlett^{1,2}, Caren Helbing¹, Helena Petrosova^{1,2}

Research objectives

- 1) Optimize a mass spectrometry imaging method to enable analysis of tadpole brains throughout metamorphosis.
- 2) Find biomolecules that characterize specific developmental stages of metamorphing tadpoles.



Background

- Mass spectrometry imaging (MSI) combines microscopic imaging with mass spectrometry to interrogate surfaces.¹
- Spatial information is particularly interesting in the brain, where different regions have different functions.²
- Amphibians are exceptional model organisms for postembryonic development.³
- Tadpole metamorphosis exhibits easily identifiable morphological changes, that we can use to link the developmental stage to biomolecular levels.⁴
- NB! These morphological changes are, however, also a challenge for histology and MSI!
- To understand if the development is disrupted, we can take advantage of the quantifiable biomolecules that characterize the developmental stages and use them as bioindicators.





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erum concentration of T3 (ng/mL) during *R. catesbeiana* metamorphosis (Gosner 31-46) measured by LC-MS/MS

One example is thyroid hormones. However, several factors make these molecules difficult to work with.⁴

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Methods and Results



Data acquisition by MALDI-timsTOF

5. Mass spectra collected at 50 µm pixel resolution





4. Data acquisition by

Bruker timsTOF fleX MALDI-2





Lipid class: Phosphoinositol

Lipid class: Flavonoid

Lipid class: Triacylglycerol

3. Norharmane matrix application:



Preliminary results: Brain ion images



ethanolamine

Ceramide phosphoinositol







First conclusions

• A mass spectrometry imaging method was developed for analysis of tadpole brains throughout metamorphosis.

Embedding and limited dissection kept the spatial integrity of the brain.

Tape transfer solved issues with tissue heterogeneity.

MSI is suitable for finding brain biomolecules that characterize specific developmental stages of vertebrate neurodevelopment.

Next steps

Further data processing using MSI software: Cardinal⁵, SCiLS and MetaboScape.

Create list of bioindicators of vertebrate neurodevelopment.

Apply biomarkers in a newly performed tadpole exposure study of the thyroid hormone system disruptor: PFOS.

I'm Thyra the Tadpole! Follow me on Instagram for updates on this project and other fun science stuff





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