Investigating the role of ethanolamine plasmalogen lipid in zebrafish brain by interdisciplinary lipidomics

Angelo Robles*, Iwan Gane, David Lamb, Emma Kenyon, Roberto Angelini

Swansea University, Medical School, Singleton Park, SA2 8PP, Swansea, United Kingdom

Introduction

An emerging field, neurolipidomics[1] aims to deliver insights into brain lipid function, thus guiding the diagnosis and treatment of neurological disorders.

Impaired lipid metabolism ↘ neurodegenerative disorders (AD) [2,3]

Ethanolamine plasmalogen (PE-p), a sub-class brain phospholipid shows a close link with AD.

↓Decreased levels PE-p ↘ cognitive decline and disease severity [4].

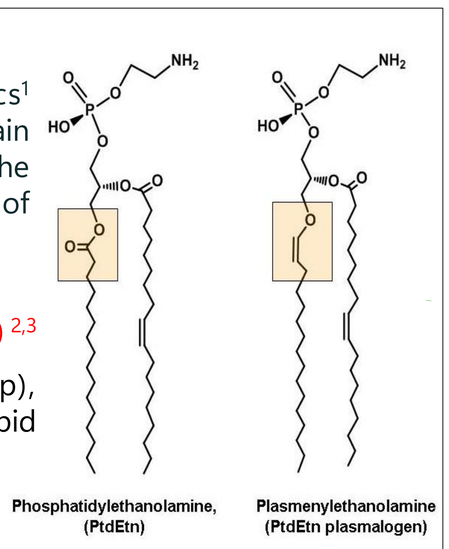


Figure 1. Structure of PE-p vs phosphatidylethanolamine. Source: Goldfine, H. (2010)

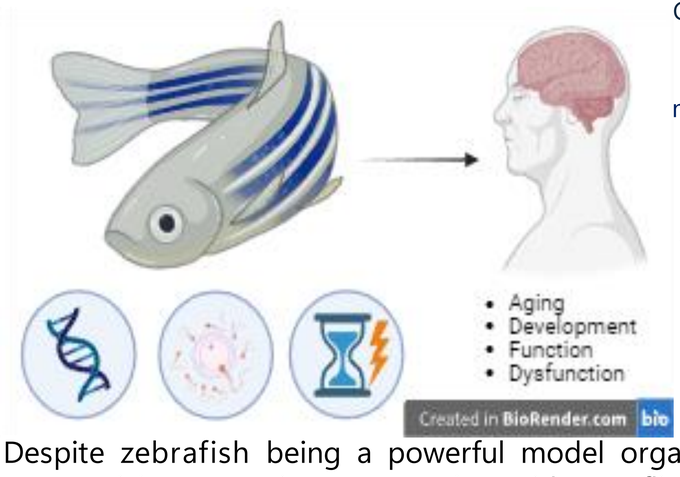


Figure 2. (Left) A model organism in neuroscience[5], Zebrafish (*Danio rerio)* utilised in study brain aging, development, function, and dysfunction[6,7].

Despite zebrafish being a powerful model organism, its lipid composition remains uncertain, with conflicting evidence regarding the presence of PE-p [8,9,10].

My research aims to uncover the role of plasmalogen in zebrafish. The current research gap in zebrafish lipidomics prompts us to utilise shotgun lipidomics to characterise the zebrafish lipidome during development and in the adult brain.

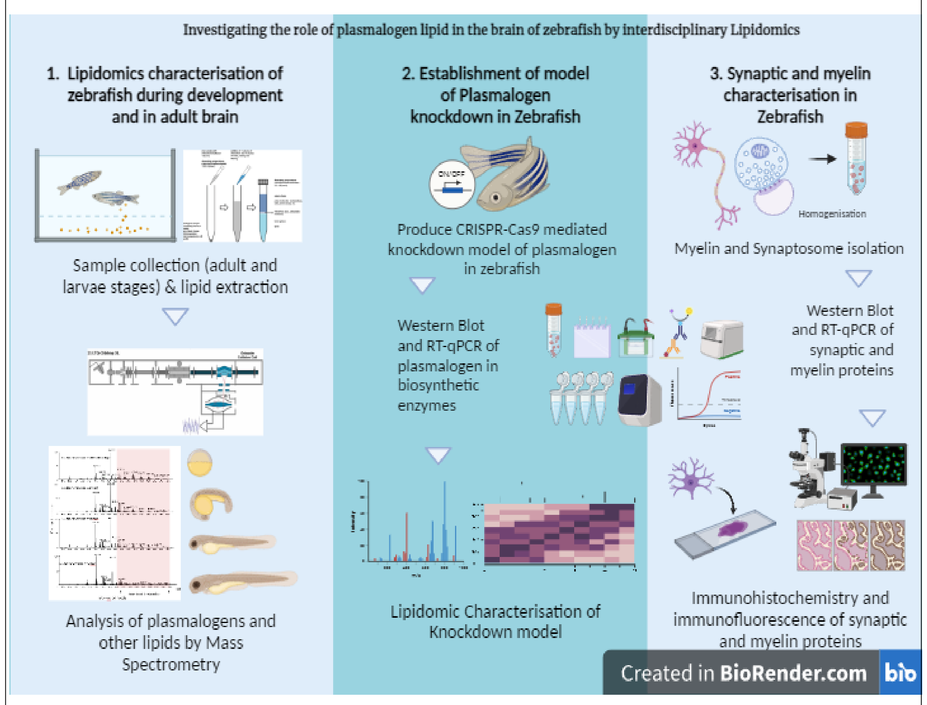
Motivation

Plasmalogens play a crucial role in cell membranes, particularly in the brain and nervous system. It is also important in inflammatory responses and in immune pathways[11,12].

However, the precise mechanisms through which plasmalogens exert their effects remains elusive.

By unravelling their role, researchers can potentially uncover new biological pathways and novel therapeutic targets with implications beyond zebrafish to human health and neurodegenerative diseases like Alzheimer's

Methods



Initial Goal: Provide conclusive evidence of PE-p presence in Zebrafish.
2nd Goal: Study the effect of brain PE-p deficiency *in vivo*.

Lipid extraction was performed on zebrafish embryos (0, 24, 48, 72 hours post fertilisation) and dissected brains from adult fish (3 months) via a modified Bligh and Dyer method.

Initially, lipid extracts were subjected to ESI-HRAM-MS/MS shotgun lipidomics via LTQ-Orbitrap XL paired with TriVersa Nanomate. Lipid modulation is postponed after method set up.

Results

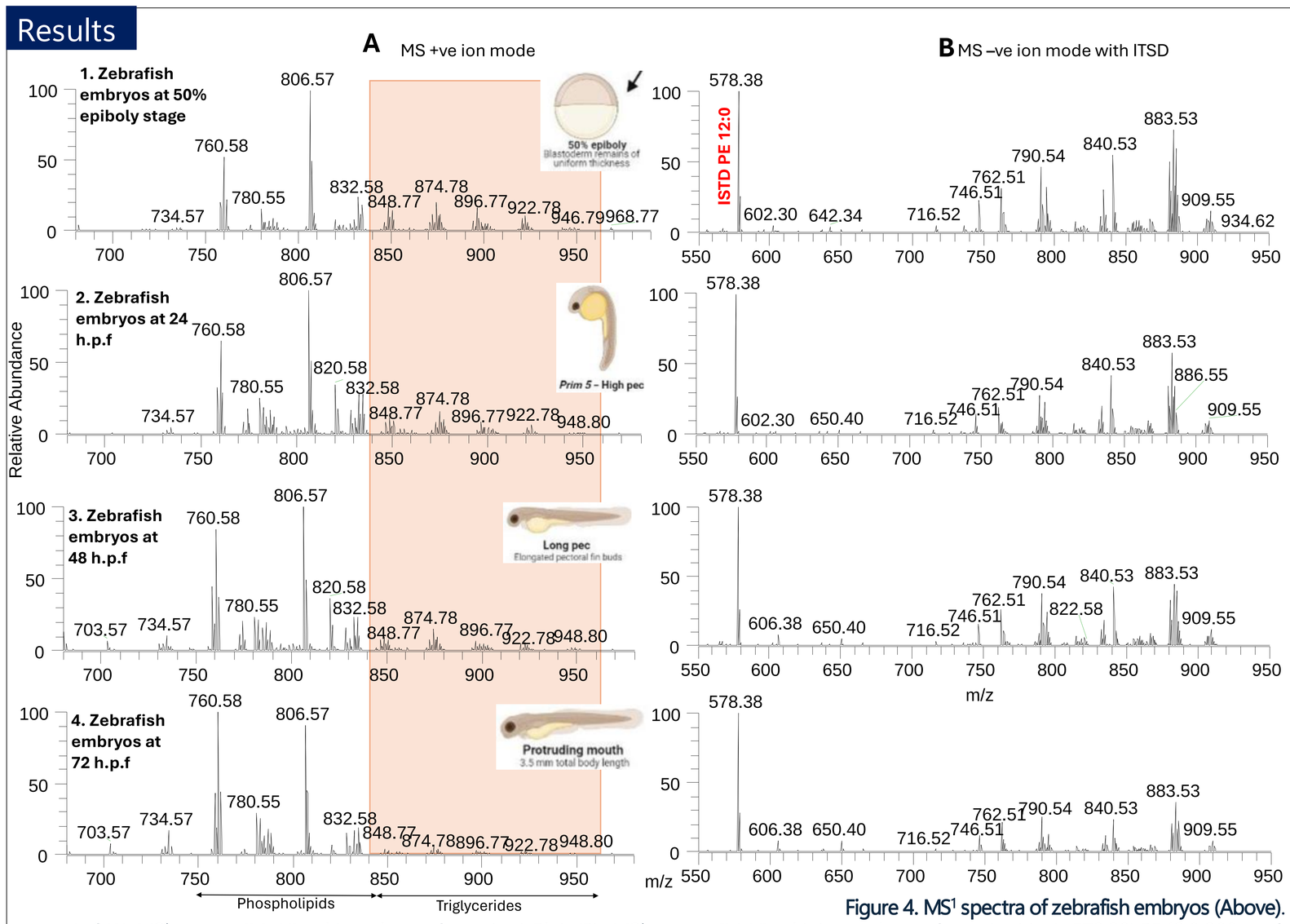


Figure 4. MS[1] spectra of zebrafish embryos (Above). Left(A)-MS[1] spectra $[M+H]^+$ without internal standard. Right(B)- MS[1] spectra $[M-H]^-$ with known internal standard (m/z 578.38) PE 12:0 300µmol. Images of Zebrafish at different stages extracted from Fig 1. of Coppola et al., 2023. . Zebrafish as a Model of Cardiac Pathology and Toxicity: Spotlight on Uremic Toxins. *International Journal of Molecular Sciences*, *24*(6), 5656. https://doi.org/10.3390/ijms24065656

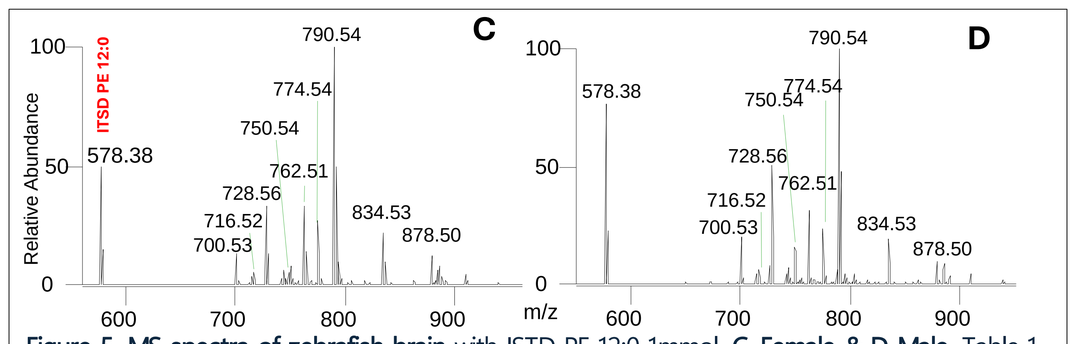


Figure 5. MS spectra of zebrafish brain with ISTD PE 12:0 1mmol. **C-Female & D-Male.** Table 1 (below) PE-P species identified.

Major Peaks:	700.53	728.56	750.54	774.54
Ether-lipid sum formula:	**PE O-34:2**	**PE O-36:2**	**PE O-34:5**	**PE O-40:7**
Fatty alkyl/ acyl lvl	PE O-16:0/18:2 PE P-16:0/18:1 PE P-18:0/16:1 PE P-20:0/14:1	PE O-16:0/20:2 PE O-18:0/18:2 PE P-16:0/20:1 PE P-18:0/18:1 PE P-20:0/16:1	PE O-16:0/22:5 PE O-18:0/20:5 PE P-16:0/22:4 PE P-18:0/20:4 PE P-20:0/18:4	PE P-18:0/22:6

Major Peaks	Ether-lipid sum formula	Fatty alkyl/ acyl lvl
716.52	PE 34:1	
746.52	PE O-38:7	PE P-16:0/22:6
762.51	PE 38:6	
790.54	PE 40:6	
794.55	PS O-38:5	
840.53	PS 40:3	
883.53	PI 38:5	
909.55	PI 40:6	

Table 2. **Major peaks of B (above)** with the known internal standard. Table 3. **Major peaks of A (below).**

Major Peaks (A)	Assignment
734.57	PC 32:0
760.58	PC 34:1
780.55	PC 36:5
806.57	PC 38:6
832.58	PC 40:7
848.77	TG 50:2
874.78	TG 52:3
896.77	TG 54:6
922.78	TG 56:7
948.80	TG 58:8

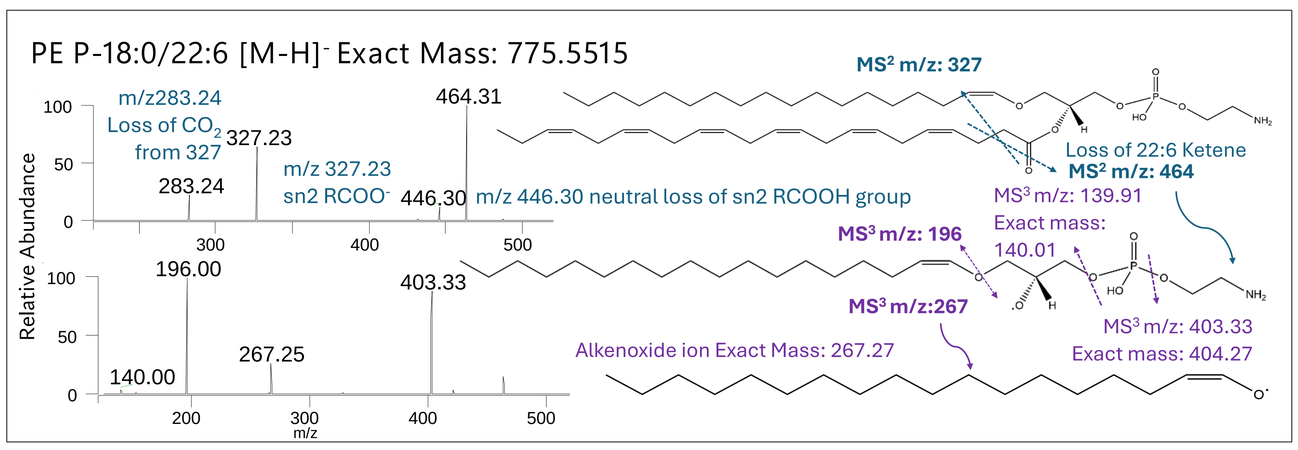


Figure 6. MSⁿ fragmentation of *m/z* 775.55 in Female zebrafish brain. High resolution MSⁿ analyses of PE (P-18:0/22:6) 774.54 its MS² and MS³ spectra ions 728.56 → 464.3 → 267.25 procured by LTQ-Orbitrap XL in tandem with TriVersa Nanomate. Pattern is congruent with Hsu (2018) showing MS² fragmentation , sn2 $RCOO^-$ at 327, loss of CO_2 from 327 at *m/z* 283, neutral loss of sn2 RCOOH group at 446, loss of 22:6 as ketene at 464 and MS³ showing the alkene oxide ion 18:1 at 267 alongside the polar head at 196 and ethanolamine phosphate ion at 139.9.

Preliminary data indicates plasmalogen PE is present in zebrafish larvae throughout development and later in the adult brain (*m/z* 775.55 PE P-18:0/22:6) warranting the use of this in vivo model for studying the role of brain plasmalogen. MS spectra $[M+H]^+$ of zebrafish embryos (A) shows a reduction of triglycerides compared to phospholipids.

Next Steps

- To confirm the presence of vinyl ether bond via mild acid hydrolysis
- Quantifying PE and PE-O against internal standard
- Data independent acquisition (DIA)
- Extend internal standard spike for global quantification of lipids

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