Functional Lipid Analysis:

a new feature integrated into the LipidOne bioinformatics platform

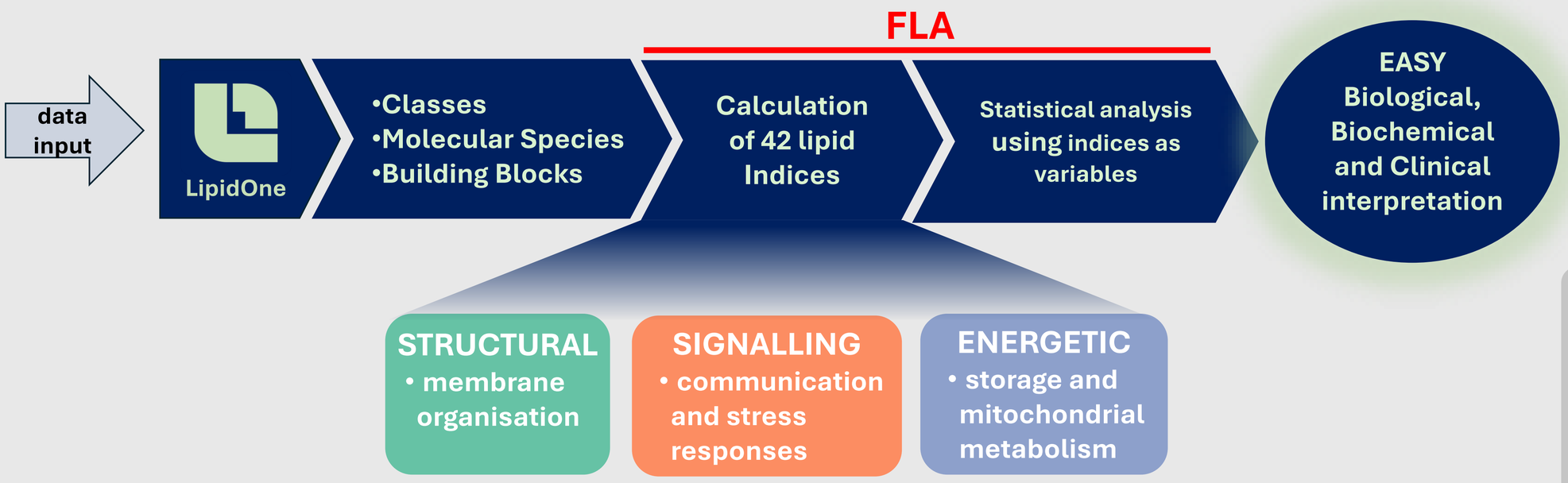
Frongia Mancini D.[1] , BR Alabed H.[2] , Koulman A.[2] , Pellegrino R.M.[1]

[1]Department of Chemistry, Biology and Biotechnology, University of Perugia, Via del Giochetto, 06123 Perugia, Italy.
[2]Core Metabolomics and Lipidomics Laboratory, Wellcome-MRC Institute of Metabolic Science, University of Cambridge, Addenbrooke's Treatment Centre, Keith Day Road, Cambridge, CB2 0QQ, UK.

HOW FLA WORKS

From numerical data to BIOLOGICAL MEANING

Functional lipid analysis (**FLA**) module* converts lipidomics data matrices into **42 literature-based functional indices** that capture changes at class, species, and building-block level. These indices are grouped into three main lipid functions: **Energy**, **Structure** and **Signaling**, and annotated with short mechanistic phrases to support biological interpretation.



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About FLA indices

- They are **literature-based** measures that link lipid variation to cellular function.
- They capture specific biological processes and translate numerical changes into **meaningful biochemical insights**.
- They are mapped to regulatory enzymes and pathways, creating a direct link from **lipidomics to proteomics and transcriptomics**.
- They offer **more robust, reproducible, and platform-independent** readouts than single-species quantitation.

Fields of application of FLA indices

CLINICAL AND TRANSLATIONAL
- biomarkers for early detection
- disease progression monitoring

SYSTEMS AND INTEGRATION
- linking lipid changes to pathways
- nutritional interventions

PRECLINICAL AND MECHANISTIC
- gene knockout/mutation studies
- metabolic phenotyping

(*) Functional Lipid Analysis via Index-Based Lipidomics Profile: A New Computational Module in LipidOne, H. B. R. Alabed, D. F. Mancini, M. Pergola, L. Romani, S. Martino, A. Koulman, R. M. Pellegrino, bioRxiv 2025.11.04.686489; doi: https://doi.org/10.1101/2025.11.04.686489

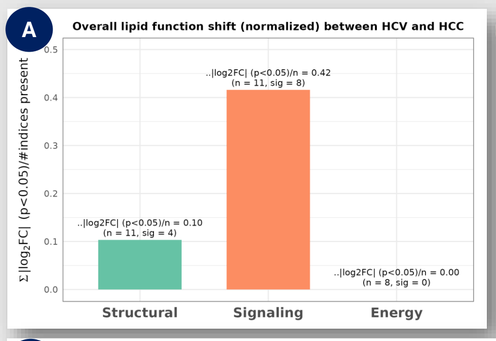
APPLICATION STUDIES

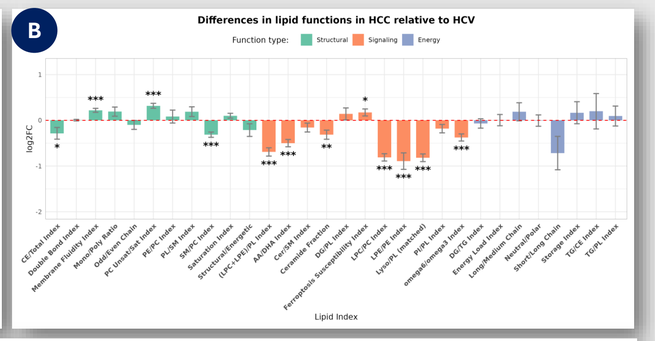
Functional Lipid Analysis shifts from chronic HCV to HCC

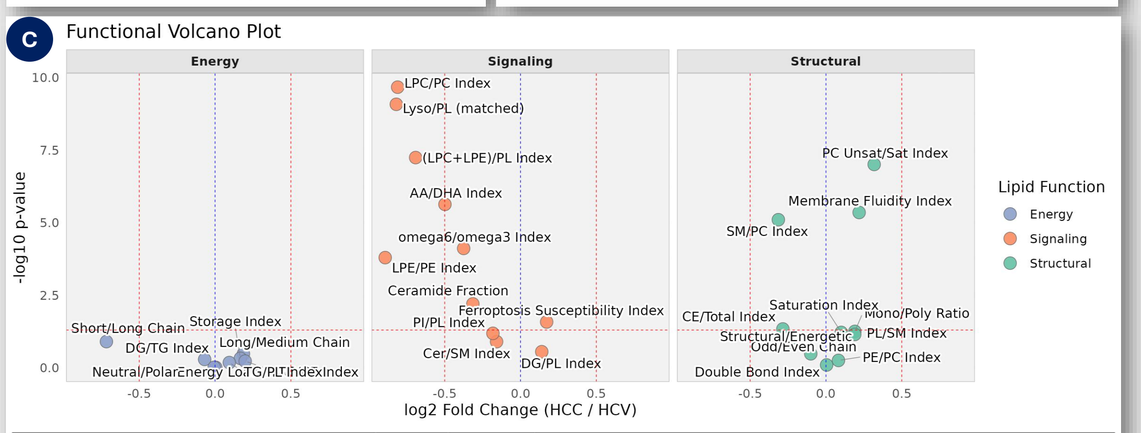
Introduction

Hepatocellular carcinoma (HCC), often linked to chronic Hepatitis C Virus (HCV), is hard to detect early, especially in AFP-negative patients. A multi-omics study (Caponigro et al., 2023) identified HCC-specific metabolic signatures, elevated short- and long-chain acylcarnitines and reduced lysophosphatidylcholines, and showed that combined metabolomic/lipidomic models outperform AFP (AUC up to 0.94). Here, we re-analyzed the lipidomics dataset using LipidOne's Functional Lipid Analysis module.

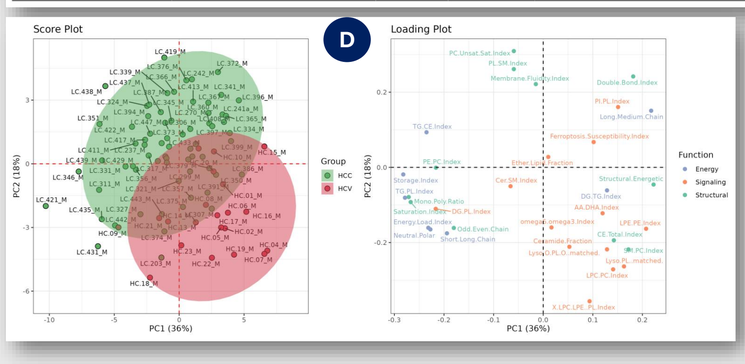
Caponigro,V. et al. (2023) Integrated plasma metabolomics and lipidomics profiling highlights distinctive signature of hepatocellular carcinoma in HCV patients. J Transl Med, 21, 918



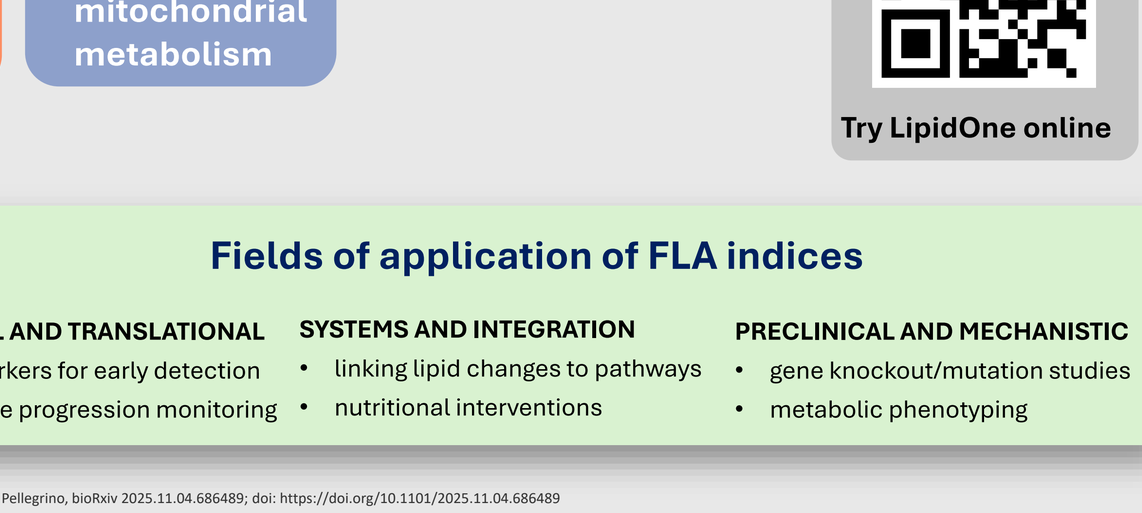




Significant Index	Function	Delta	log2FC	pvalue	Biological Interpretation of Lipidomic Indices
LPC/PC Index	Signaling	HCC << HCV	-0.810	2.14e-10	Lower LPC/PC ratio indicates reduced phospholipid hydrolysis.
Lyso/PL (matched)	Signaling	HCC << HCV	-0.819	8.32e-10	Lower lysophospholipids relative to chain-matched PC+PE; consistent with reduced PLA2 activity or efficient reacylation.
(LPC+LPE)/PL Index	Signaling	HCC << HCV	-0.692	5.71e-08	Lower lysophospholipid content suggests membrane stability or low PLA2 activity.
LPE/PE Index	Signaling	HCC << HCV	-0.892	1.6e-04	Reduced LPE/PE ratio may indicate stable mitochondrial lipid composition and reduced mitochondrial signaling.



Variable	Function	p_value	AUC	Cohen's d	Power % (p<0.001)
Lyso/PL (matched)	Signaling	1.99e-09	0.95	2.30	100.0
LPC/PC Index	Signaling	6.33e-10	0.95	2.35	100.0
(LPC+LPE)/PL Index	Signaling	1.22e-08	0.91	1.85	100.0
Lyso-O/PL-O (matched)	Signaling	1.07e-06	0.91	1.64	99.9
AA/DHA Index	Signaling	1.34e-06	0.88	1.59	99.8
LPE/PE Index	Signaling	8.3e-05	0.85	1.24	93.8
omega6/omega3 Index	Signaling	1.04e-04	0.83	1.17	89.8
SM/PC Index	Structural	8.95e-06	0.83	1.33	97.0
PC Unsat/Sat Index	Structural	4.15e-07	0.81	1.16	89.2
Membrane Fluidity Index	Structural	2.7e-05	0.77	1.06	79.3
Mono/Poly Ratio	Structural	4.09e-04	0.74	0.85	50.1
Ceramide Fraction	Signaling	0.011	0.74	0.75	35.1
Storage Index	Energy	0.002	0.72	0.78	40.3
TG/CE Index	Energy	0.002	0.71	0.73	32.9
Saturation Index	Structural	0.002	0.71	0.81	45.1
TG/PL Index	Energy	0.009	0.69	0.65	22.3
CE/Total Index	Structural	0.026	0.67	0.63	19.8
PI/PL Index	Signaling	0.036	0.67	0.57	14.3
Ether Lipid Fraction	Signaling	0.020	0.65	0.57	14.6
Structural/Energetic	Structural	0.047	0.64	0.55	12.5



Results and discussion

We re-analysed the plasma lipidomics dataset of Caponigro et al. (HCV-positive subjects with and without HCC) using the Functional Lipid Analysis (FLA) module of LipidOne. Instead of inspecting hundreds of individual species, FLA condenses the lipidome into mechanism-based indices that track signalling, structural and energetic functions and provides a short biochemical interpretation for each index.

A Across all panels, the transition from chronic HCV to HCC appears dominated by changes in lipid signalling.

B Most significantly altered indices belong to the signalling category, with a consistent depletion of multiple lysophospholipid-based indices in HCC. This pattern is compatible with impaired phospholipase-mediated membrane remodelling. Structural indices show more modest shifts, such as reduced PC unsaturation and altered membrane fluidity, whereas energy-storage indices remain largely preserved.

C **E** The functional volcano plot and summary table highlight a small set of indices with large effect size, high AUC and adequate statistical power, including ether-lysophospholipid, LPE/PE (mitochondrial inner-membrane) and ceramide-axis indices.

D PCA on functional indices captures a larger fraction of variance (PC1 = 35.9%) and yields clearer separation between HCV and HCC than PCA on molecular species (PC1 = 20.3%), indicating that indices both dampen single-species noise and enhance interpretability.

F The accompanying protein network maps these key indices to their predicted regulatory enzymes, pointing to coordinated dysregulation of glycerophospholipid and arachidonic-acid metabolism and providing a mechanistic bridge from lipidomics to proteomics.

Conclusions

In this case study, Functional Lipid Analysis confirms the lysophosphatidylcholine depletion originally reported in HCC and reframes it within a broader functional taxonomy linking membrane remodelling, inflammation and organelle turnover. Because FLA uses ratio-based, mechanism-grounded indices with attached statistics (effect size, AUC, power), it delivers portable, biomarker-ready readouts that are particularly valuable for early, AFP-independent detection of HCC and for translating untargeted lipidomics into biologically meaningful hypotheses.

University of Perugia
A.D. 1308
unipg
DEPARTMENT OF CHEMISTRY, BIOLOGY AND BIOTECHNOLOGY

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