LipidCreator: A new bridge between targeted and non-targeted LC-MS/MS-based lipidomics

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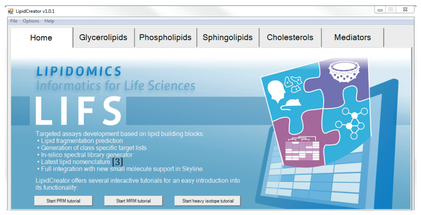
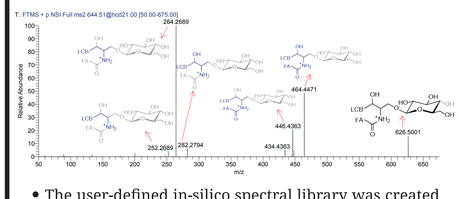
Introduction

Skyline, a powerful software primarily designed for proteomics applications, was successfully extended to targeted LC-MS/MS based lipidomics [1, 2]. The proof-of-principle study provided a systematic workflow for the straightforward method design and analysis of selected reaction monitoring (SRM) data in lipidomics based on lipid building blocks. To strengthen the LC-MS/MS-based lipidomics workflow and to provide a user-friendly interface for lipid researchers, we developed a native tool named "**LipidCreator**".



Methods

- LipidCreator was written in C# to be compatible with Skyline.
- Four types of building blocks (backbone, head group, fatty acyl, long chain base) were used to create neutral or isotope labeled lipids.
- The pre-defined fragmentation of lipid classes was obtained from synthetic lipid standards and high-resolution MS2 analysis, e.g. HexCer 18:1;2/12:0



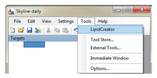
- The user-defined in-silico spectral library was created on-the-fly with LipidCreator.

Results

1. Targeted lipidomics workflow

A) Open LipidCreator from Skyline.
B) Create a list of lipid species of interest.
C) Select the type of product ions, or add user-defined product ions.
D) Review the list for precursor and product ion pairs.
E) Import the list directly into Skyline. Then export MS methods from Skyline.

A)



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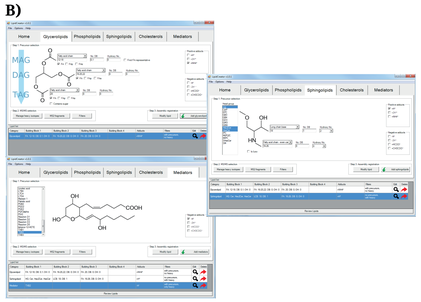

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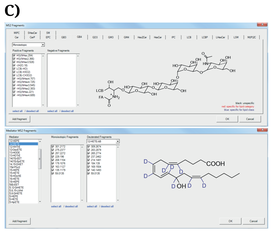

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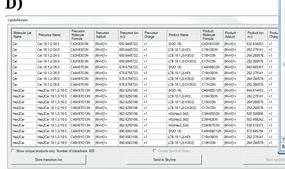

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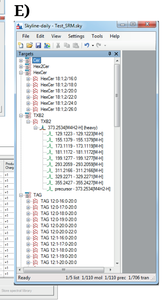
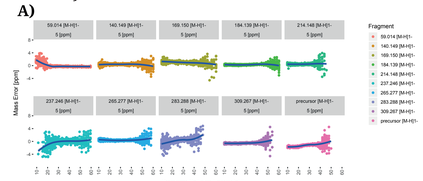


2. Create in-silico spectral library

Each lipid standard was measured with multiple collision energies in one run to calculate the prediction models of fragment intensities, e.g. 12-HETE-d8:
A) mass error distribution for each fragment.
B) normalize obtained intensity with total ion scan.
C) scan relative intensity for each fragment.
D) predicted relative intensity after model training.
E) residuals between predicted and actual measured intensity.

A)



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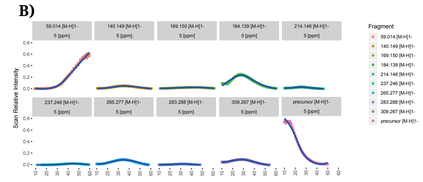

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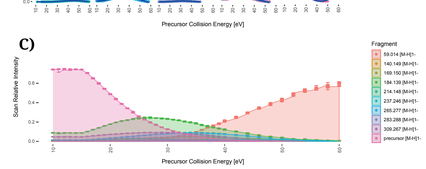

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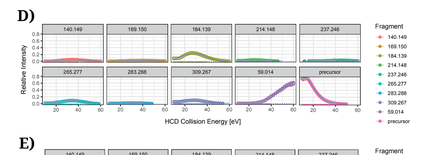

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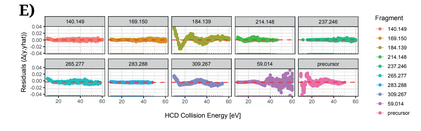
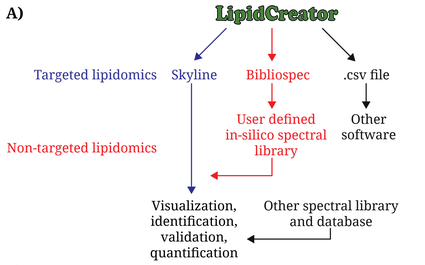


3. Extend to non-targeted workflow

A) LipidCreator provides different workflows.
B) PRM (parallel reaction monitoring) data analysis with targeted transition list.
C) DIA (data independent acquisition) data analysis with in-silico spectral library generated from LipidCreator.

A)



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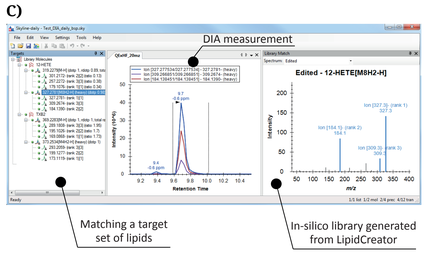
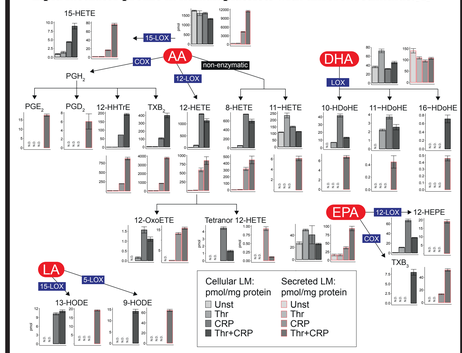


Application

Lipid mediator profile in human platelets with different stimulus.[4]



Analyzed mediators in platelets and their corresponding secretome level. Bar graphs with blank framed colors display mediators in platelets while bars with red frame indicate mediators in the corresponding secretome. The absolute quantities are reported in pmol/mg protein.

Summary

- LipidCreator provides targeted assays for 5 lipid categories, ~60 lipid classes, 92 mediators, by using neutral or isotope labeled building blocks.
- The predefined fragmentation patterns were visualized with individual structure images.
- The in-silico spectral library for lipids establishes the connection between targeted and non-targeted analysis.

References

[1] MacLean B., MacCoss M. J., et al. Bioinformatics 2010, 26 (7), 966-8.
[2] Peng B., Ahrends, R. J. Proteome Res. 2016, 15, 291–301.
[3] Pauling J.K., Hermansson M., et al. PLoS One 2017, 12(11) : e0188394.
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