

Optimisation of quantitative proteomics combining trapped ion mobility mass spectrometry and data independent acquisition

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INTRODUCTION

Over the last years, bottom-up proteomics has improved by developing mass spectrometers (MS) which are able to analyse with higher resolution, higher accuracy as well as significantly increased scanning speed. Nowadays, it is getting more important to know the exact quantity of proteins to allow a better understanding of biological systems. With the combination of trapped ion mobility to time-of-flight MS (tims-TOF) in combination with parallel accumulation-serial fragmentation (PASEF), the analysis in proteomics was revolutionized. The coupling of TIMS to TOF adds a new separation dimension based on the collision-cross section (CCS) of ions. And PASEF allows higher sequencing speed and sensitivity due to a serial set-up.

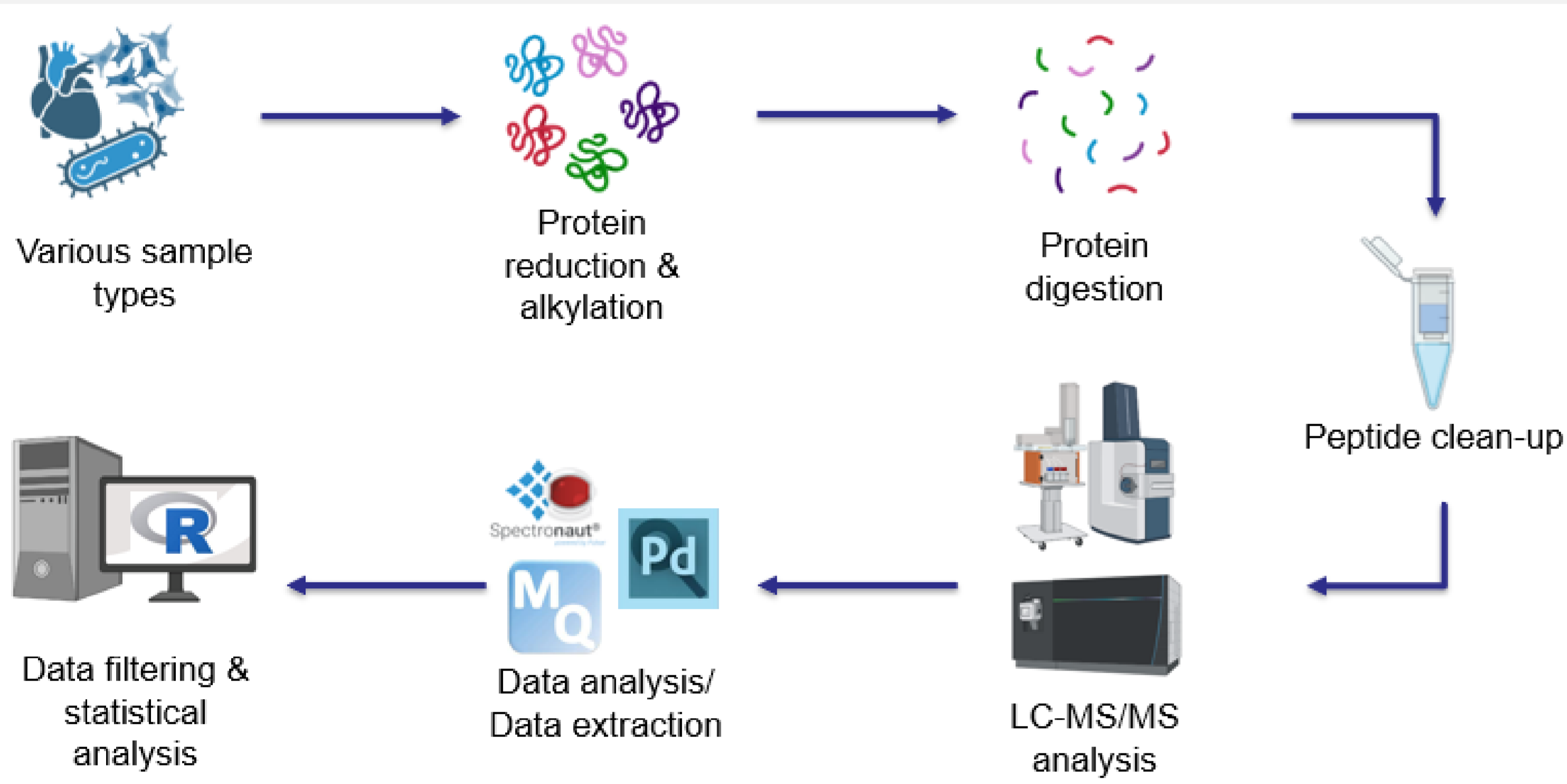


Fig 1: Common proteomics workflow for the bottom-up approach created with BioRender.

CONCEPT

The presented thesis aimed to optimise a data acquisition and analysis method to detect proteins with high quantification accuracy, reproducibility, and high protein coverage. For that, in total 12 different parameter combinations on the tims-TOF Pro 2 from Bruker, as well as the data analysis workflow in R were evaluated. Optimisation of five key parts of the tims-TOF Pro 2 ensure all necessary parts of the instrument. Those key parameters were visualized in Fig. 2.

- Funnel 1 RF (400 – 500 Vpp)
- Ramp time & accumulation time (100 – 150 ms)
- Number of mobility windows (1 – 2)
- Multipole RF (200 – 500 Vpp)
- Mass width (10 – 40 Da)

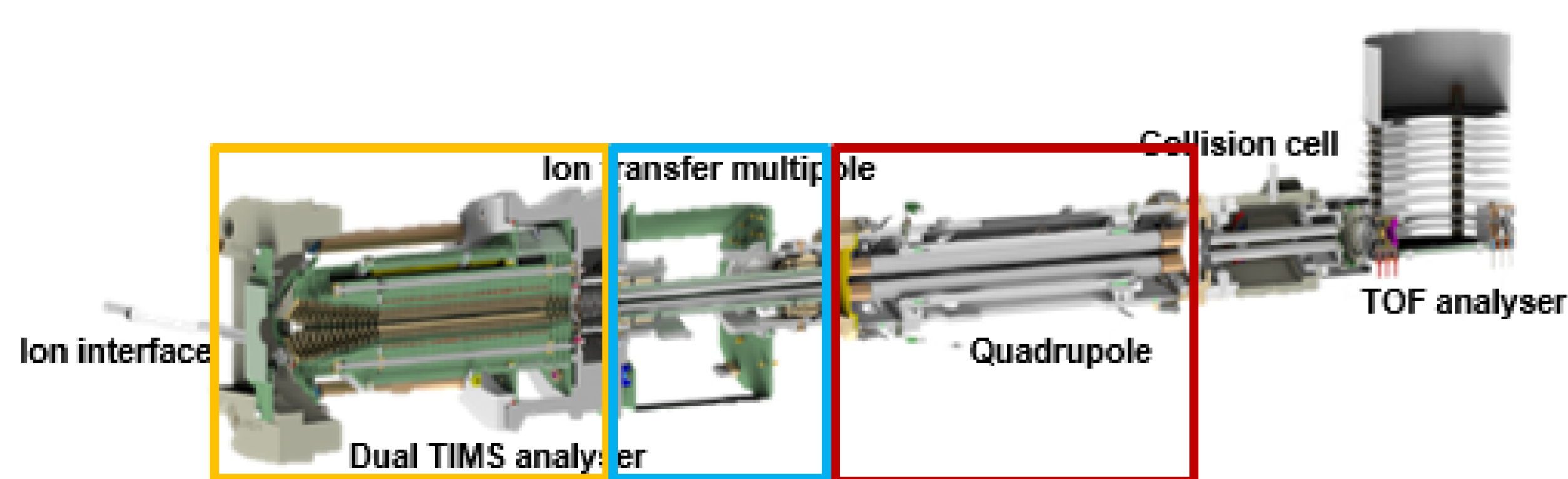


Fig 2: timsTOF Pro 2 set-up with marked areas where optimisations were done [1].

For optimisation of the data analysis workflow, it was tested 20 different data filtering combinations, six normalisation workflows and five aggregation workflows. The analysis was challenged by using complex hybrid-proteome samples consisting of human, yeast and *E. coli* proteome. The evaluation of the workflow was done with four parameters:

- the log₂ fold-change (L2FC) -> Accuracy
- the coefficient of variation (CV) -> Reproducibility
- number of quantified elution groups (EG)
- number of quantified protein

RESULTS

With the optimized workflow it was possible to quantify 11'835 proteins. The accuracy was 20 % higher compared to the standard workflow. There was no decrease regarding the reproducibility.

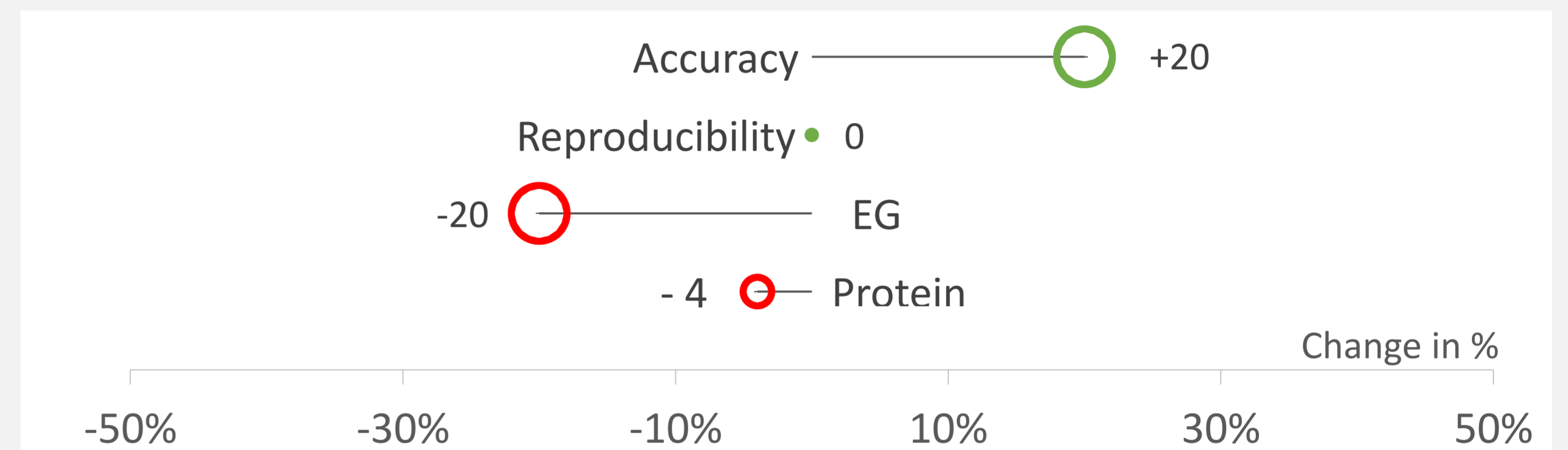


Fig 3: Results of the optimized workflow for the 60-min LC-gradient.

The newly developed method was used to evaluate the influence of different statins on THP-1 cells. It was possible to detect subtle differences between the compounds which usually influence 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA). For Simvastatin a clear up-regulated effect was detected.

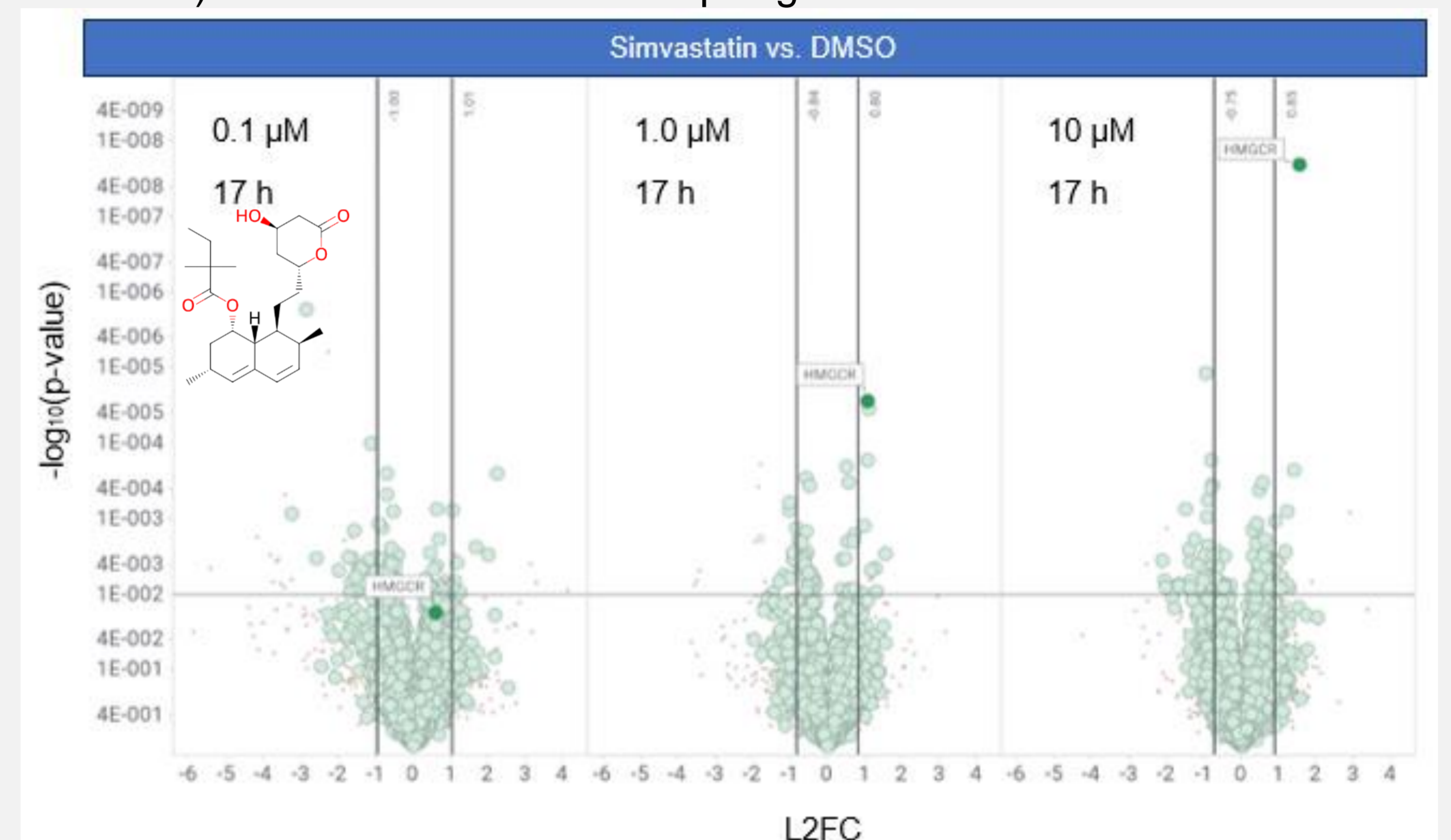


Fig 4: Volcano plot for compound Simvastatin.

Pravastatin, showed an up-regulating effect on HMG-CoA at 10 µM.

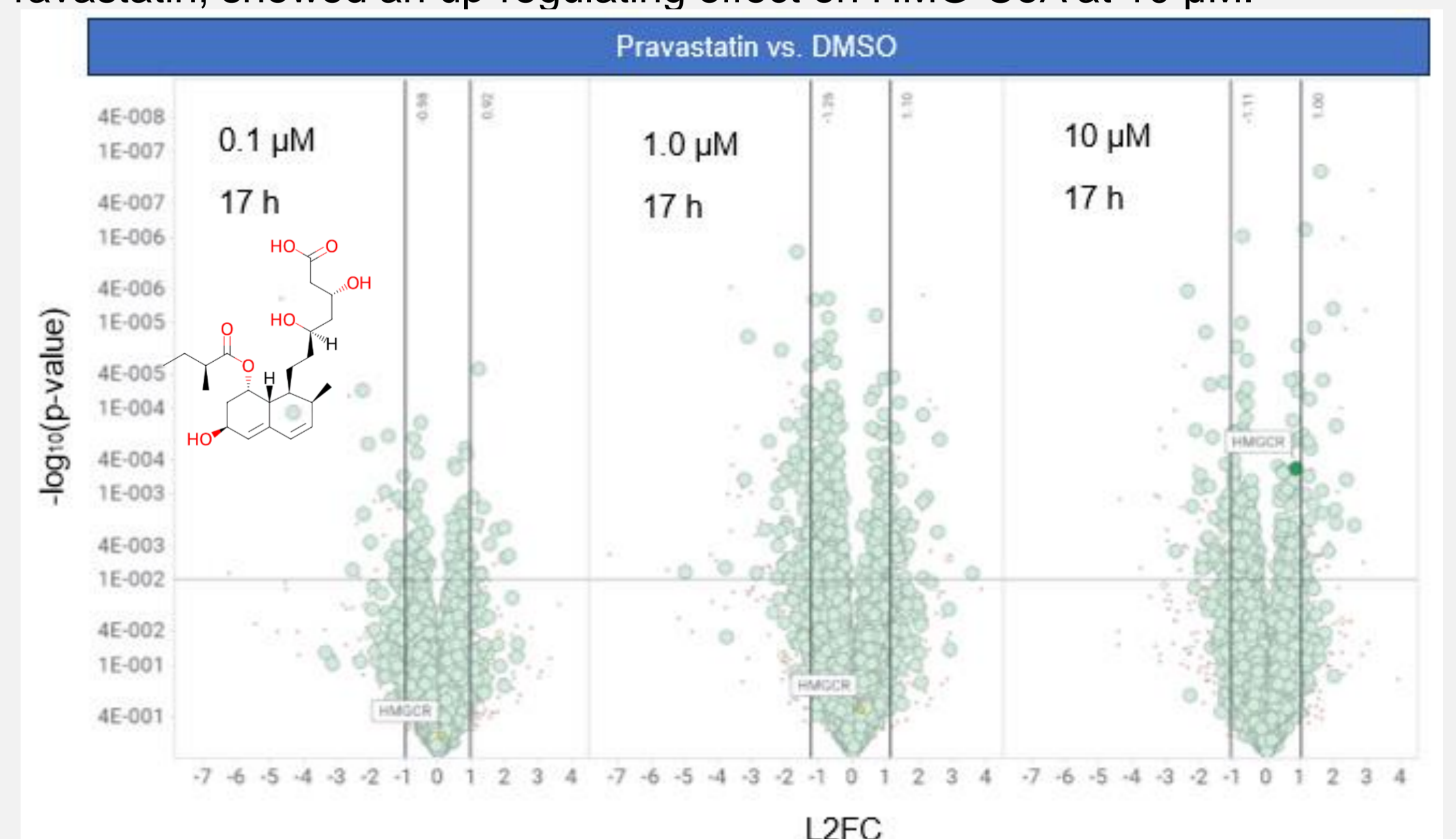


Fig 5: Volcano plot for compound Pravastatin.

CONCLUSION & OUTLOOK

It was possible to optimize the bottom-up proteomics workflow and achieving higher accuracy with same reproducibility compared to the standard workflow. The proteome coverage slightly decreased; however, the results will be more precise for further analyses. As well it was demonstrated that subtle differences can be detected for cell-line experiments.

REFERENCES

[1] MEIER, F., PARK, M. A. & MANN, M. 2021. Trapped Ion Mobility Spectrometry and Parallel Accumulation-Serial Fragmentation in Proteomics. *Mol Cell Proteomics*, 20, 100138

