

Introduction

- lipids play an **important role** on cellular level, i.e., **phospholipids** as main components for **membrane stabilization**^[1]
- phospholipids** consist of **nonpolar acyl chains** with varying chain length and number of double bonds as well as **different phosphate-based subclasses** (Figure 1)
- due to the **structural diversity** and huge number, **lipid quantitation** remains analytically **challenging** and requires both **powerful analytical techniques** and **tailored software solutions**

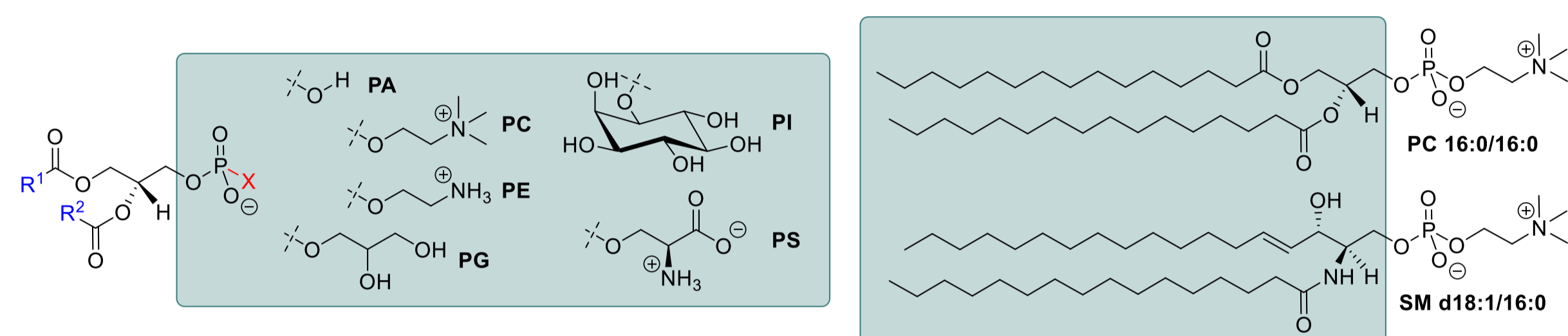


Figure 1: Schematic overview of the glycerophospholipid classes phosphatidic acid (PA), phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylglycerol (PG), phosphatidylinositol (PI) and phosphatidylserine (PS), as well as comparison of the phospholipid classes sphingomyelin (SM) and phosphatidylcholine (PC) based on selected lipid species. Undefined residues are shown in blue.

Method

- a **4D-Lipidomics approach** utilizing trapped ion mobility spectrometry (TIMS) hyphenated into an **LC-TIMS-MS/MS workflow** was developed (Figure 2)
- lipid class separation** by hydrophilic interaction liquid chromatography (HILIC) for orthogonal and **unambiguous lipid assignment** (Figure 3)
- parallel reaction monitoring-parallel accumulation-serial fragmentation (**prm-PASEF**) for **targeted 4D-Lipidomics** due to its **reproducible and comprehensive MS2 scheduling**^[2] (Figure 4)
- for **absolute quantitation**, a stable isotope labelled **internal standard (IS) per lipid class** was used with external calibration
 - Sample:** NIST SRM 1950 plasma (Matyash extraction^[3]), Splash Lipidomix internal standard mixture
 - LC separation:** iHILIC Fusion(+) column, 18 min gradient
 - Data acquisition:** fimsTOF flex, ESI negative, 500 ms ramp time, prm-PASEF

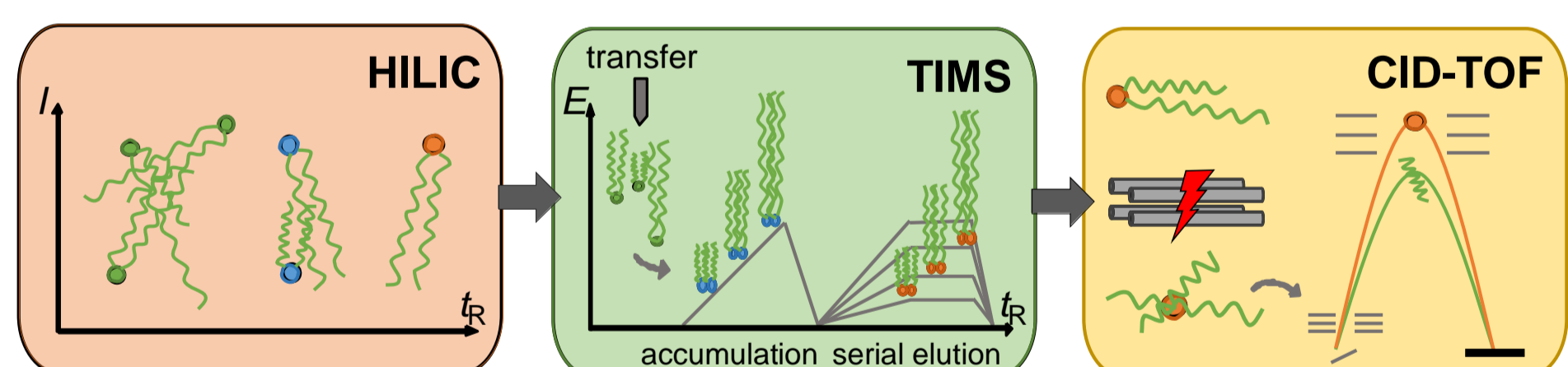


Figure 2: Schematic overview of the utilized HILIC-TIMS-MS/MS workflow for targeted and quantitative 4D-Lipidomics.

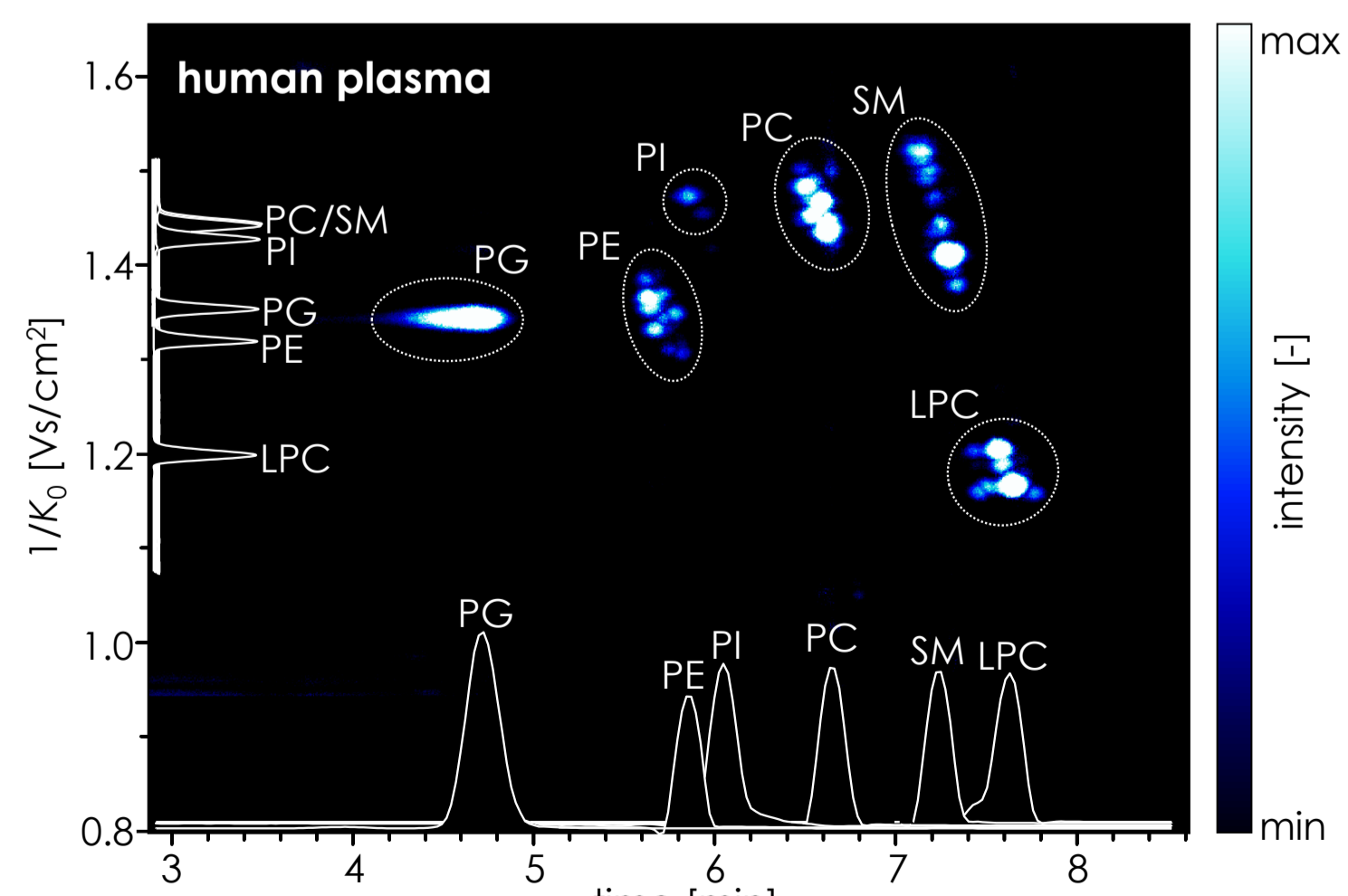


Figure 3: Heatmap of the human plasma phospholipids using HILIC-TIMS-PASEF. In white, normalized EICs and EIMs of lipid standards are illustrated.

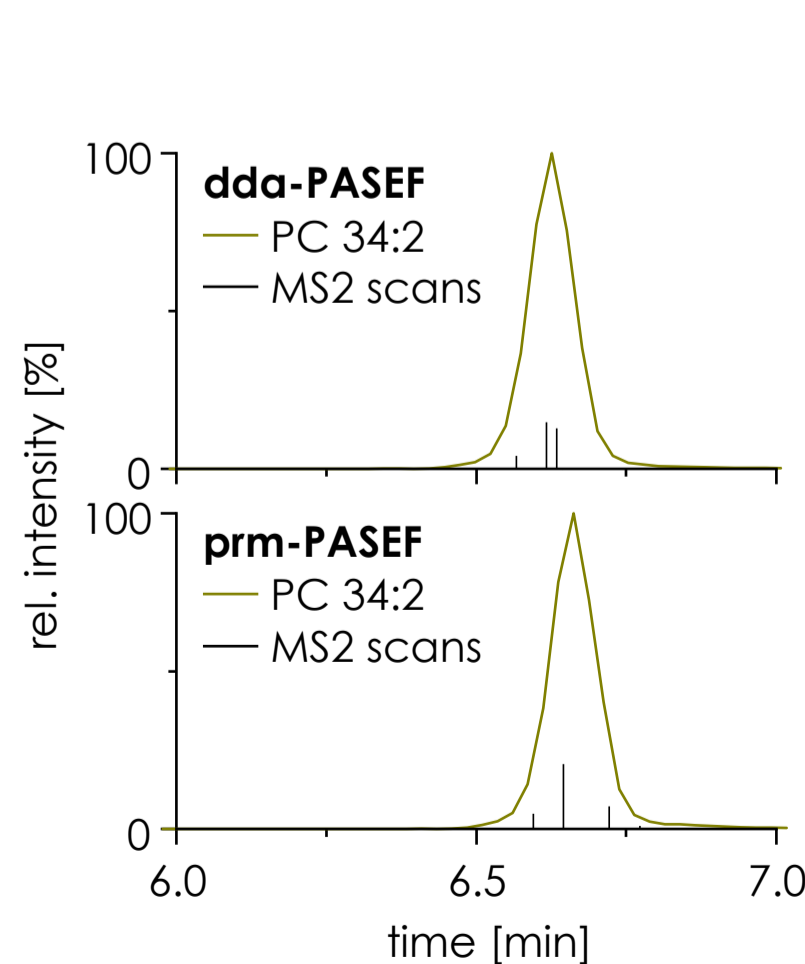


Figure 4: MS2 scheduling using dda-PASEF and prm-PASEF exemplified for PC 34:2.

This poster in a nutshell:

- precise lipid quantitation with high confidence based on HILIC-TIMS-PASEF using prm-PASEF
- user-friendly workflow by combination of MetaboScape 2024b and TASQ 2024b software solutions



Figure 5: Scheme of the developed workflow for high-confidence lipid quantitation by prm-PASEF.

Workflow

HILIC-TIMS-MS/MS screening

- using **4D-Lipidomics**, a **deep lipidome coverage** with **high confidence annotation** based on retention time, isotope pattern, collisional cross section and fragmentation behavior is obtained in **MetaboScape software**

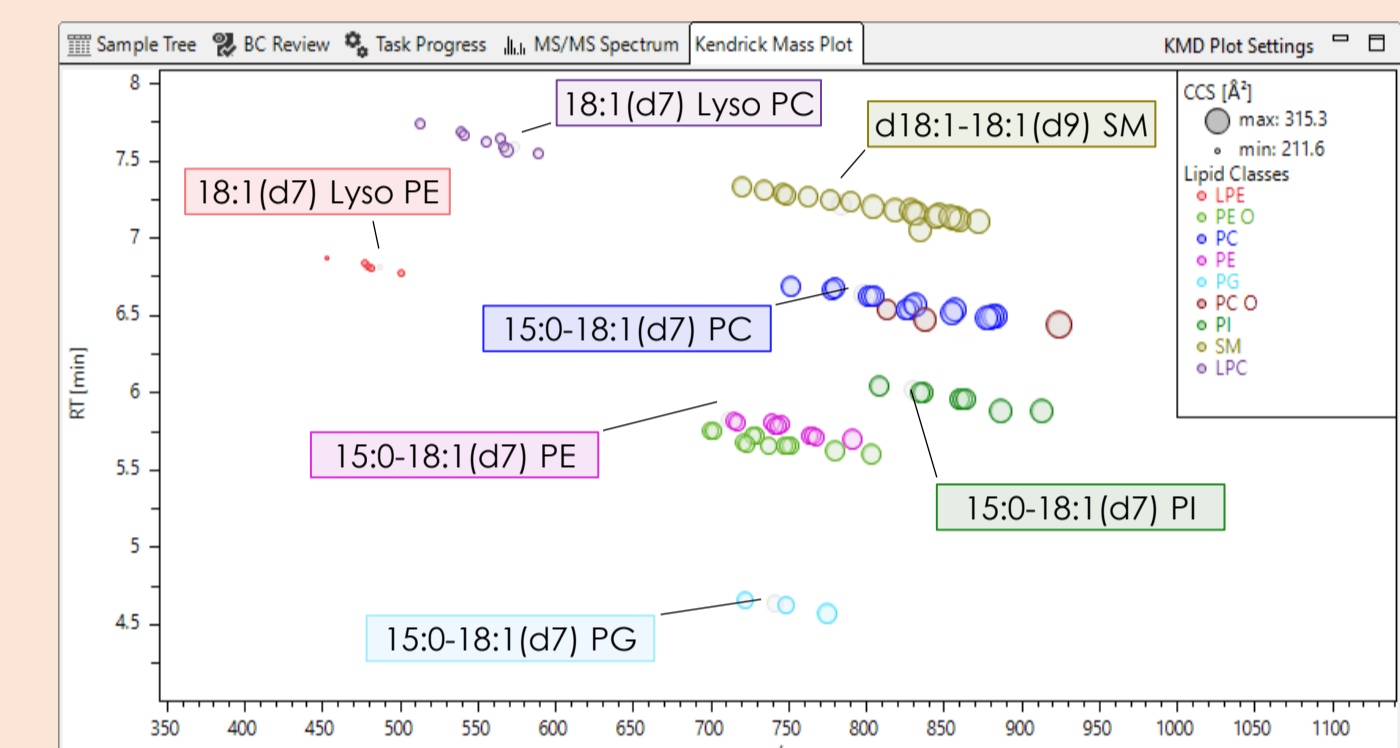


Figure 6: Survey plot of the annotated lipid species in MetaboScape.

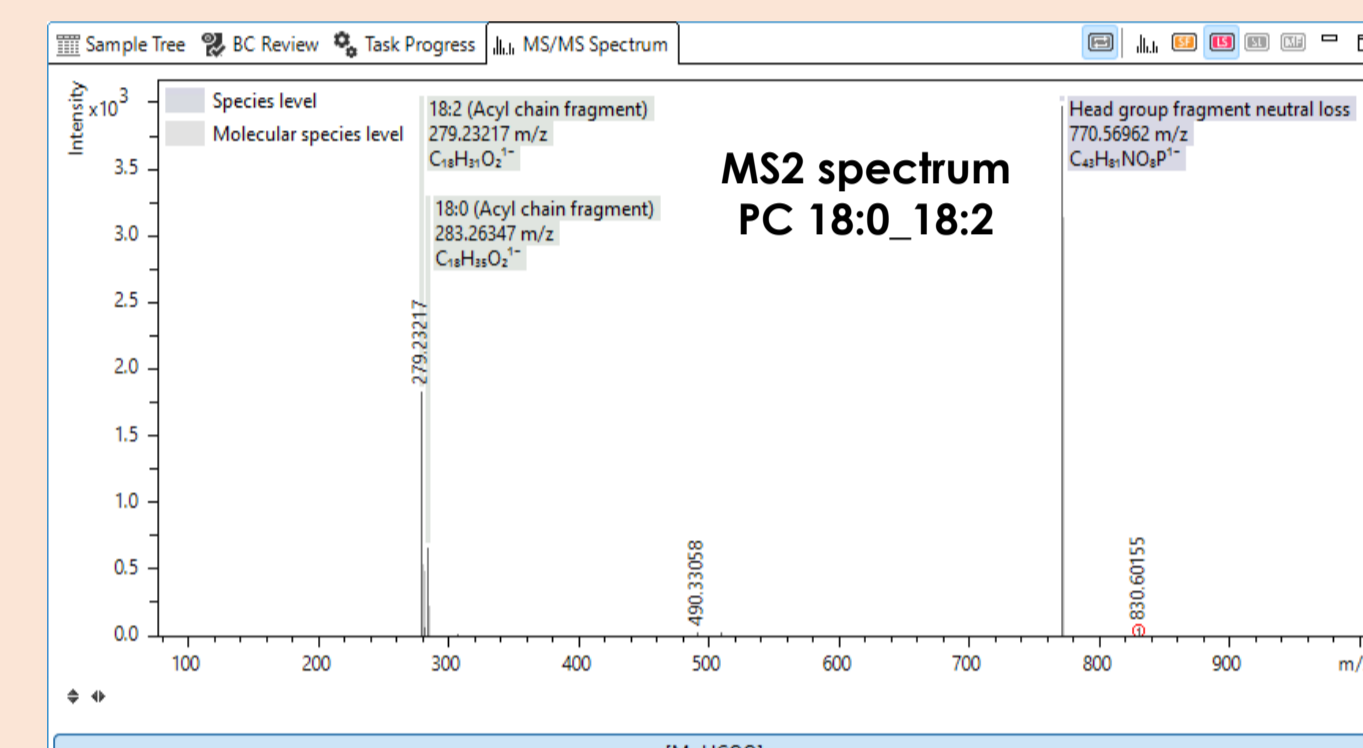


Figure 7: MS2 spectrum of PC 18:0_18:2 with highlighted transitions by the rule-based lipid annotation module in MetaboScape.

Quantitative data processing in TASQ

- annotated **lipid species** are transferred to the **targeted/quantitative TASQ software** including **lipid species transitions** that support the **reliability in lipid quantitation** on species or molecular species level
- this transfer also facilitates the **seamless generation of a prm-PASEF method** in TASQ software

Analyte	Formula	Mass [Da]	Reg.D	RT expected [min]	RT obs [min]	RT error [min]	RT width [min]	CCS [Å²]	CCS error [Å²]	CCS ratio [%]	Number formulae
18	LPE 18:2	C ₃₆ H ₇₀ O ₆	472.3855	6.60	6.50	0.10	0.40	213.84	7.00	2.00	2
20	LPE 20:4	C ₃₈ H ₇₀ O ₆	501.3855	6.70	6.50	0.20	0.40	217.88	7.00	2.00	2
21	PC 14:0_16:0	C ₃₈ H ₇₀ O ₆	705.5300	6.60	6.50	0.10	0.40	283.83	7.00	2.00	2
22	PC 16:0_16:0	C ₃₈ H ₇₀ O ₆	735.5300	6.60	6.50	0.10	0.40	289.89	7.00	2.00	2
23	PC 16:0_18:1	C ₃₈ H ₇₀ O ₆	731.5480	6.60	6.50	0.10	0.40	287.80	7.00	2.00	2
24	PC 16:0_18:1	C ₃₈ H ₇₀ O ₆	735.5480	6.60	6.50	0.10	0.40	293.84	7.00	2.00	2
25	PC 16:0_18:2	C ₃₈ H ₇₀ O ₆	731.5620	6.60	6.50	0.10	0.40	291.87	7.00	2.00	2
26	PC 16:0_20:3	C ₃₈ H ₇₀ O ₆	765.5770	6.50	6.50	0.00	0.40	296.46	7.00	2.00	2
27	PC 18:0_20:4	C ₃₈ H ₇₀ O ₆	765.5620	6.50	6.50	0.00	0.40	292.23	7.00	2.00	2
28	PC 18:0_18:2	C ₃₆ H ₇₀ O ₆	735.5480	6.60	6.50	0.10	0.40	292.22	7.00	2.00	2
29	PC 18:0_20:3	C ₃₈ H ₇₀ O ₆	811.0000	6.50	6.50	0.00	0.40	302.89	7.00	2.00	2
31	PC 18:0_20:4	C ₃₈ H ₇₀ O ₆	806.9855	6.50	6.50	0.00	0.40	301.24	7.00	2.00	2

Figure 8: Overview of the transferred lipids from MetaboScape to TASQ including transitions exemplified for PC 18:0_18:2.

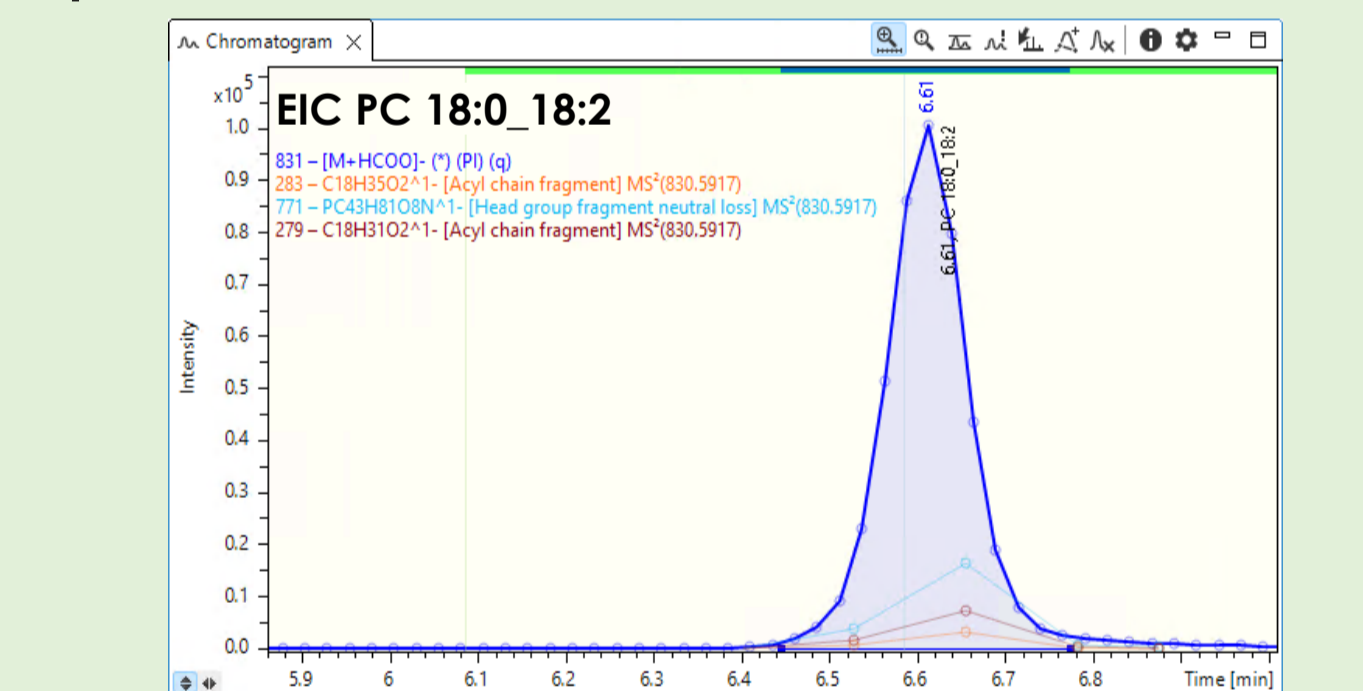


Figure 9: EIC of PC 18:0_18:2 including lipid species transitions by prm-PASEF for reliable quantitation using the developed workflow.

Targeted prm-PASEF acquisition

- using prm-PASEF, **mobility-resolved MS2 scans** are focused on lipids of interest leading to **high data quality**

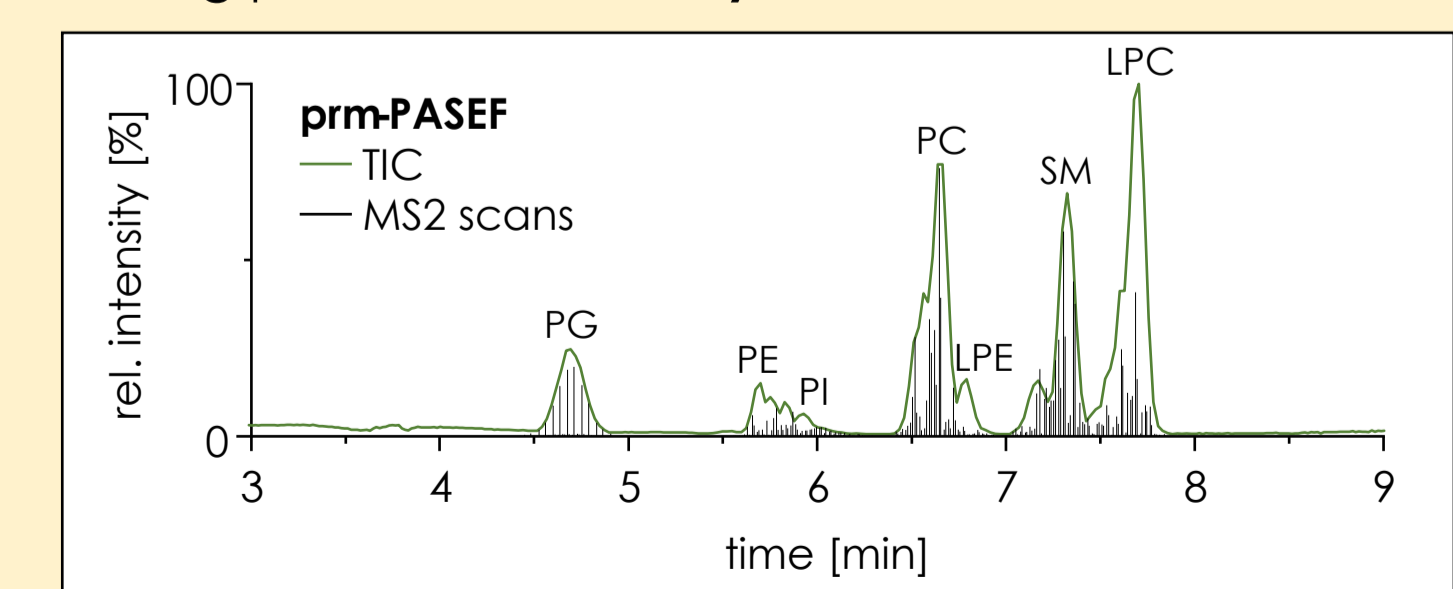


Figure 10: TIC of the NIST plasma lipid extract with highlighted MS2 scans focusing on lipids of interest using prm-PASEF.

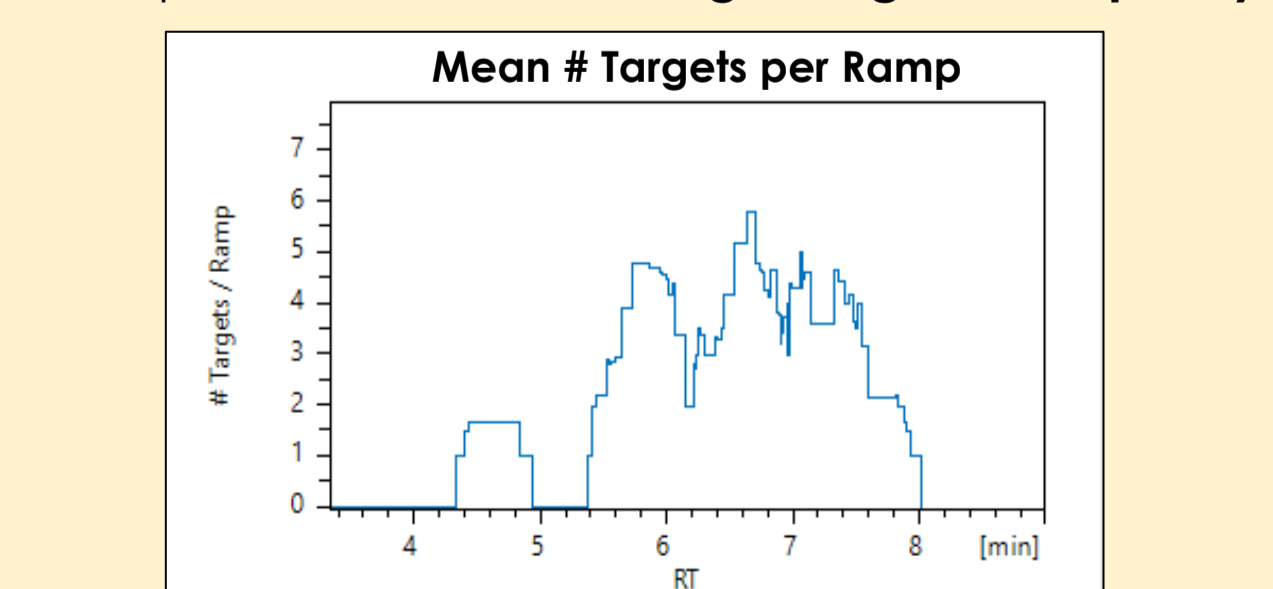


Figure 11: Overview of the number of mobility-resolved targets as a function of the retention time for each prm-PASEF scan.

Lipid quantitation

- lipid class separation** by HILIC-MS/MS favors **quantitation** using one IS per lipid class
- however, this **coelution favors** also **isobaric type-II overlaps** that result from the natural isotopic pattern of **lipid species** with an **additional double bond** ($\Delta m/z = 2$ Da; Figure 12a+b)
- using **high ramp times**, a **TIMS separation** of isobaric **type-II overlaps** is obtained (Figure 12c)

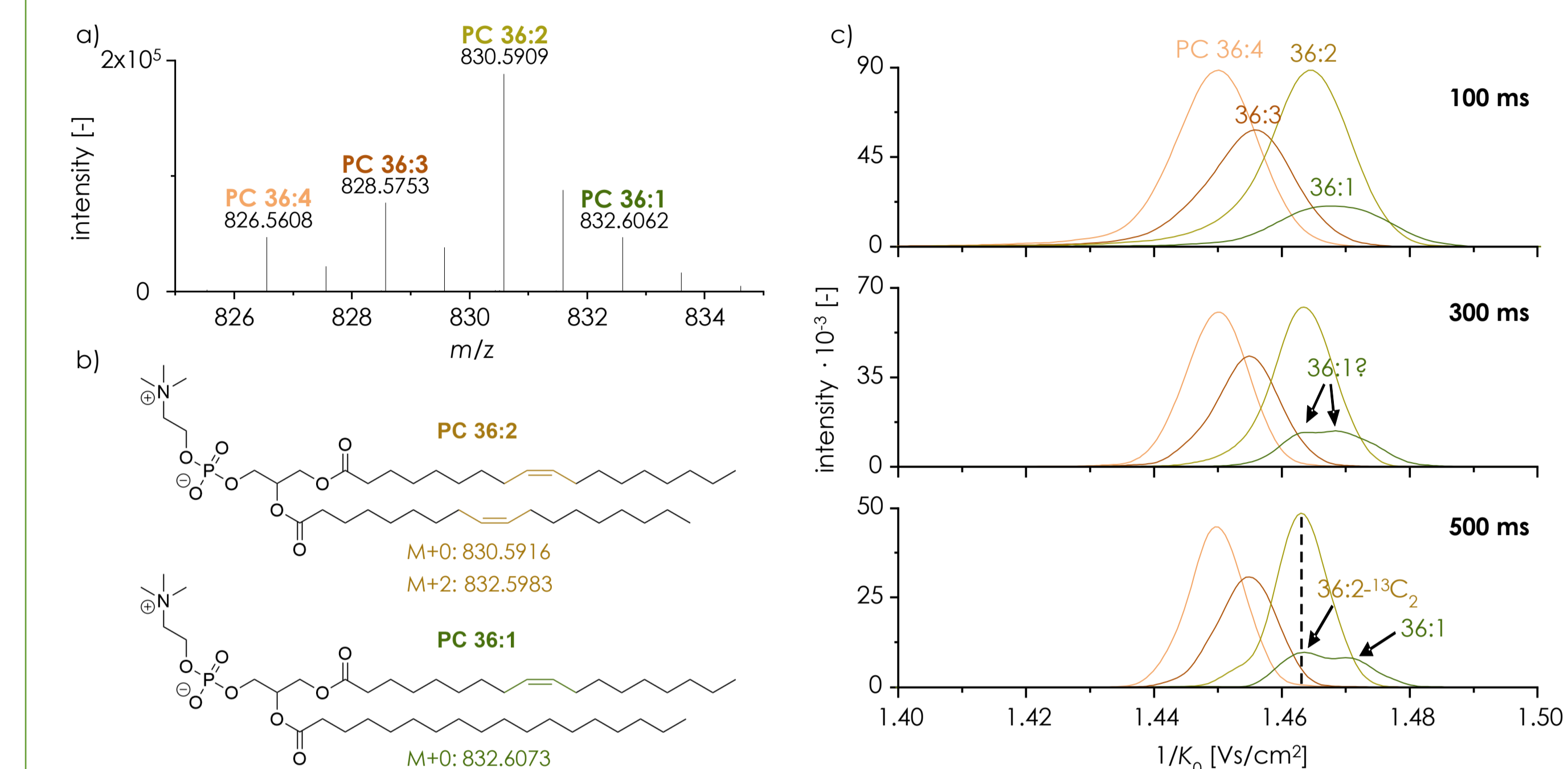


Figure 12: a) Scheme of isobaric type-II overlaps in MS based on the natural isotopic pattern of lipids, b) exemplified for PC 36:1. c) TIMS separation of PC species in human plasma of a homologous series with varying double bonds and varying ramp times.

- for **accurate quantitation** of non-baseline **TIMS resolved type-II overlaps**, i.e., PC 36:1 and SM d34:0 (Figure 13), we recommend the use of the **M+1 signal for quantitation** in TASQ
- thus, **TIMS improved the quantitation accuracies** compared to conventional type-II correction factors for **PC 36:1** and **SM d34:0** by **74 %** and **42 %**, respectively (Figure 14)
- our developed workflow served for a **precise and user-friendly quantitation** of **> 80 lipid species** with **high confidence** in **lipid quantitation** on species or molecular species level

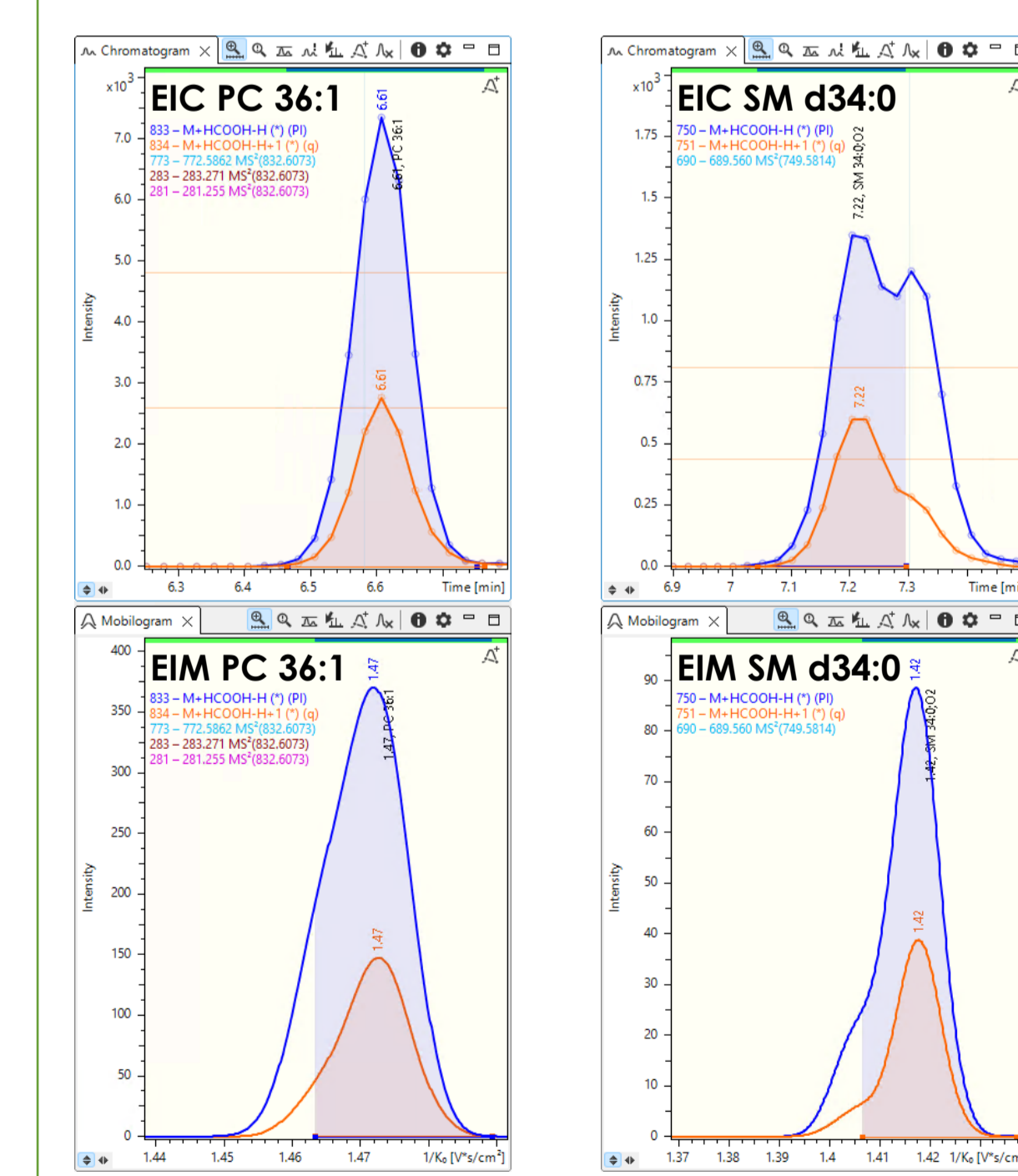


Figure 13: EICs and EIMs of PC 36:1 and SM d34:0 in TASQ showing the TIMS (and HILIC) separation of isobaric type-II interferences.

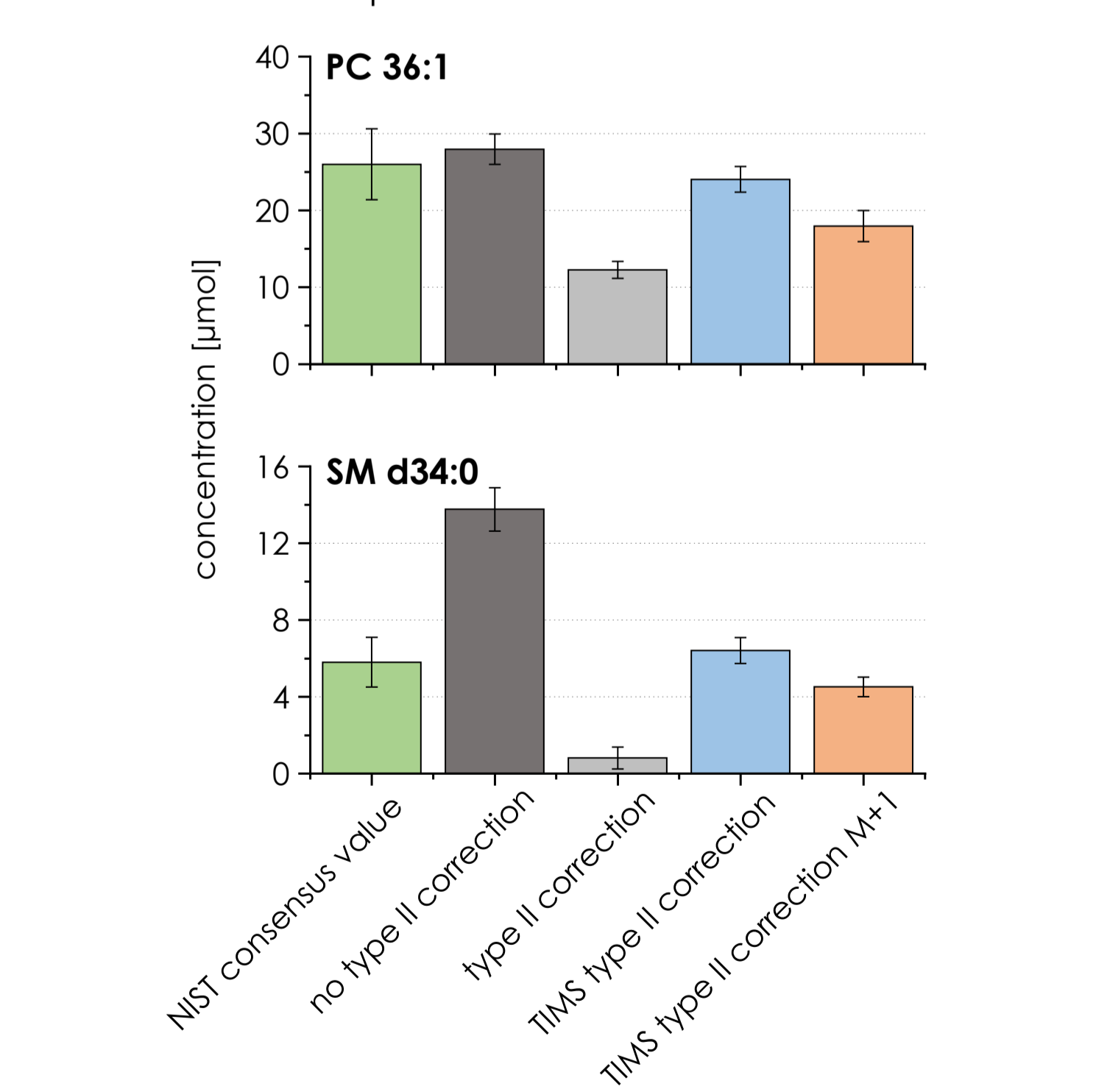


Figure 14: Quantitation accuracies for PC 36:1 and SM d34:0 in the NIST reference plasma using different correction factors. TIMS type-II correction improves reproducibility of the consensus values. By selection of the M+1 signal, accuracy is improved by up to 74 %, compared to conventional type-II correction factors.

Literature

- van Meer, G.; Voelker, D. R.; Feigenson, G. W. *Nat. Rev. Mol. Cell. Bio.* **2008**, *9* (2), 112–124.
- Rudt, E.; Feldhaus, M.; Margraf, C. G.; Schlehner, S.; Schubert, A.; Heuckeroth, S.; Karst, U.; Jeck, V.; Meyer, S. W.; Korf, A.; Hayen, H. *Anal. Chem.* **2023**, *95*, 9488.
- Matyash, V.; Liebisch, G.; Kurzchalia, T. V.; Shevchenko, A.; Schwudke, D. *J. Lipid Res.* **2008**, *49* (5), 1137–1146.

Acknowledgement

The authors would like to thank Wen Jiang (HILICON AB, Umeå, Sweden) for providing the iHILIC Fusion column for HILIC measurements.

Conflict of Interest

The contributing authors VJ, NK, CM, IK, HN are currently employed by Bruker Daltonics GmbH and Co. KG. Bruker is a supplier of commercial TIMS instruments.