Ultra-sensitive nanoLC-MS using second generation micro pillar array LC technology with Orbitrap Exploris 480 and FAIMS PRO to enable single cell proteomics

Karel Stejskal[1,3,4,§], Jeff Op de Beeck[2,§], Gerhard Dürnberger[1,3,4], Paul Jacobs[2] and Karl Mechtler[1,3,4*]

[1] IMBA - Institute of Molecular Biotechnology of the Austrian Academy of Sciences, Dr. Bohr Gasse 3, A-1030 Vienna, Austria. [2] PharmaFluidics, Technologiepark-Zwijnaarde 82, B-9052 Gent, Belgium. [3] IMP - Institute of Molecular Pathology, Campus-Vienna-Biocenter 1, A-1030 Vienna, Austria. [4] Gregor Mendel Institute of Molecular Plant Biology of the Austrian Academy of Sciences, Dr. Bohr Gasse 3, A-Vienna, Austria.

[§] KS and JB will contribute equal to the manuscript.

ABSTRACT

In the light of the ongoing single-cell revolution, scientific disciplines are combining forces to retrieve as much relevant data as possible from trace amounts of biological material. For single cell proteomics, this implies optimizing the entire workflow from initial cell isolation down to sample preparation, liquid chromatography (LC) separation, mass spectrometer (MS) data acquisition and data analysis. To demonstrate the potential for single cell and limited sample proteomics, we report on a series of benchmarking experiments where we combine LC separation on a new generation of micro pillar array columns with state-of-the-art Orbitrap MS/MS detection and High-Field Asymmetric Waveform Ion Mobility Spectrometry (FAIMS). This dedicated limited sample column has a reduced cross section and micro pillar dimensions that have been further downscaled (inter pillar distance and pillar diameter by a factor of 2), resulting in improved chromatography at reduced void times. A dilution series of a HeLa tryptic digest (5-0.05 ng/µL) was used to explore the sensitivity that can be achieved. Comparative processing of the MS/MS data with Sequest HT, MS Amanda, Mascot and SpectroMine pointed out the benefits of using Sequest HT together with INFERYS when analyzing samples amounts below 1 ng. 2855 proteins were identified from just 1 ng of HeLa lysate, hereby increasing detection sensitivity as compared to a previous contribution by a factor well above 10. By successfully identifying 1486 proteins from as little as 250 pg of HeLa tryptic digest, we demonstrate outstanding sensitivity with great promise for use in limited sample proteomics workflows.

INTRODUCTION

During the last few years, the sensitivity of LC-MS/MS instrumentation has evolved to a level where consistent identification and quantification of proteins from single cells has become feasible [1–5]. As opposed to LC-MS/MS analysis of proteins originating from bulk cell populations, proteome analysis of a single or a few selected cells allows attributing biological characteristics to individual cells. It can also provide crucial and unbiased insights in the role that specific cell types play in biological processes [6]. As already observed in bulk proteomics samples, recent studies demonstrate modest correlation between transcriptomic and proteomic expression levels obtained from single cells [1,7]. This highlights the complementary nature and pertinence of LC-MS/MS based proteome profiling at the single cell level.

In contrast to single cell analysis at the genomic and transcriptomic level, single cell proteome analysis cannot rely on techniques that allow amplifying trace amounts of protein [8]. Therefore the

entire proteomics workflow needs to be optimized carefully to achieve ultrasensitive analysis and near loss-less processing of protein samples [9–11]. Recent breakthroughs in the field of low input LC-MS/MS based proteomics have been obtained in parallel by several independent groups, each using a unique approach to tackle the challenges associated with comprehensive single cell proteome analysis [1,3,12]. The key aspects to the successful implementation seem to be a combination of miniaturized and automated sample preparation methods with ultrasensitive LC-MS/MS analysis performed at very low LC flow rates (≤ 100 nL/min), additional ion mobility separation and the latest generation of hybrid or tribrid MS-MS instruments. As from the inception of nanoelectrospray in the 90s by Mann et al. [13], the major leaps in sensitivity that can be achieved by combining true nanoliter per minute flow rates with electrospray ionization have been a driving force in the practice of MS based proteomics. However, robust operation at very low flow rates (≤ 100 nL/min) is still a big challenge and requires either highly specialized LC systems that are not widely available, or custom column configurations.

Building further upon a previous contribution where we reported on the benefits of using perfectly ordered micro pillar array based nano HPLC columns for low-input proteomics [14], we now present an ultrasensitive LC-MS/MS based proteomics workflow at standard nano flow rates (250 nL/min). By combining outstanding chromatographic performance of a novel dedicated micro pillar array column type with High-Field Asymmetric Waveform Ion Mobility Spectrometry (FAIMS) Pro technology, the latest generation of Orbitrap MS systems and optimized search strategies, we manage to achieve more than a 10 fold increase in detection sensitivity on the protein level as compared to Stadlmann et al. in 2019 [14]. The potential of this approach for single cell proteome profiling is demonstrated by analyzing a dilution series of HeLa cell tryptic digest from 5 ng down to 50 pg, resulting in consistent identification of 2855 proteins from 1 ng of HeLa tryptic digest.

EXPERIMENTAL

To investigate the potential gain in sensitivity that can be achieved for low input proteome profiling, the micro pillar array column was operated with an Ultimate™ 3000 RSLCnano system with ProFlow™ technology (Thermo Fisher Scientific) and coupled to an Orbitrap Exploris™ 480 mass spectrometer (Thermo Fisher Scientific) equipped with a FAIMS pro interface (Thermo Fisher Scientific). The dedicated limited sample micro pillar array column (PharmaFluidics) has a total length of 50 cm and is filled with 2.5 µm diameter non porous silicon pillars that have been positioned at a distance of 1.25 µm in an equilateral triangular grid (Supplementary information figure 1). The stationary phase morphology has been optimized to deliver maximal performance for low input reversed phase liquid chromatography, which is discussed more extensively in the supplementary information.

Sample preparation

Thermo Scientific™ Pierce™ HeLa Protein Digest Standard (P/N 88328) was obtained from Thermo Scientific™ in lyophilized form and used as test sample for all measurements. 20 µg of lyophilized peptide material in glass vial was reconstituted to a concentration of 100 ng/µL in LC/MS grade water with 0.1% (v/v) formic acid (FA). For the final dilution to required concentration, peptide solution was diluted directly in autosampler vials with a glass insert (Thermo Scientific™, P/N 500212). The solvent used to perform dilutions was 0.001% (w/v) polyethylene glycol (PEG 20000, Sigma Aldrich, P/N 83100) in LC/MS grade water with 0.1% (v/v) formic acid (FA). To investigate the effect of PEG addition to the sample stability, samples were diluted to 250 pg/µl in autosampler vial with glass insert. Injection was performed over a period of 24 h after dilution at 4 h intervals. A HeLa dilution series was finally prepared with PEG

in the sample solvent and samples were injected as technical quadruplicates, starting with the lowest concentration (0.25 ng/µl) and ending with the highest concentration (5 ng/µL).

LC configuration

The column was placed in the column oven compartment and maintained at a constant temperature of 40°C throughout the entire experiment. A shortened fused silica capillary (20 µm ID x 360 µm OD, length 40 cm, P/N 6041.5293, Thermo Fisher Scientific) was used to connect the column inlet to the Ultimate™ 3000 RSLCnano autosampler injection valve, configured to perform direct injection of 1µL volume sample plugs (1µL sample loop – full loop injection mode). Separation was achieved with stepped linear solvent gradients, all performed at a fixed flow rate of 250 nL/min with various durations of 30, 45 and 60 minutes. Organic modifier content (Acetonitrile acidified with 0.1% v/v formic acid) was first increased from 0.8 to 16% in 24.5, 35.75 and 47 min, then increased from 16 to 28% in respectively 7.5, 11.25 and 15 min and finally ramped from 28 to 78% in 5 min. Mobile phase composition was kept at high organic (78% Acetonitrile acidified with 0.1% v/v formic acid) for 5 minutes to wash the column after which column re-equilibration was performed at low organic (0.8% Acetonitrile acidified with 0.1% v/v formic acid) for 40 minutes.

Efficient transfer of peptides from the LC column to the mass spectrometer was achieved by connecting the column outlet union to a PepSep sprayer 1 (PepSep, P/N PSS1) equipped with 10 µm ID fused silica electrospray emitter with integrated liquid junction (PepSep, P/N PSFSELJ10) using a custom made fused capillary with nanoConnect (PepSep, 20 µm ID x 360 µm OD, length 15 cm). A grounded connection was provided between the column outlet union and the grounding pin at the back of the RSLCnano system to prevent electrical current leaking to the LC column. An electrospray voltage of 2.3 kV was applied at the integrated liquid junction of the emitter which was installed on a Nanospray Flex™ ion source (Thermo Fisher Scientific).

MS Acquisition

The mass spectrometer was operated in positive mode with the FAIMS Pro interface. compensation voltage was set at -50V to remove singly charged ions. Data dependent acquisition (DDA) was performed using the following parameters. MS1 resolution was set at 120k with a normalized AGC target 300 % (3e6), a maximum inject time was set to Auto and a scan range of 375 to 1200 m/z. For MS2, resolution was set at 60k with a normalized AGC target of 75 % (7.5e4), maximum inject time of 118 ms. Top 10 abundant precursors (charge state 2-5) within an isolation window of 2 m/z were considered for MS/MS analysis. Dynamic exclusion was set at 120s, mass tolerance of ±10 ppm was allowed, and the precursor intensity threshold was set at 5e3. For precursor fragmentation in HCD mode, a normalized collision energy of 30% was used. Whereas technical replicates were not evaluated for initial gradient length and sample stability optimization, technical quadruplicates were run for the in-depth analysis of the HeLa tryptic digest dilution series (5 to 0.25 ng).

Data analysis

MS/MS spectra from raw data acquired at various concentrations of HeLa tryptic digest were imported to Proteome Discoverer (PD) (version 2.5.0.400, Thermo Scientific). All database search engines were operated with identical parameter settings. First MS/MS spectra were recalibrated in the PD node “Spectrum Files RC” using the human SwissProt database (*Homo sapiens*; release 2019_06, 20339 sequences, 11360750 residues) and a database of common

contaminants (372 sequences, 145187 residues). Recalibration was performed for fully tryptic peptides applying an initial precursor tolerance of 20 ppm and a fragment tolerance of 0.1 Da. Carbamidomethylation of cysteine was set as a fixed modification in the recalibration step. Database searches were performed using the same FASTA databases as described above. Trypsin was specified as proteolytic enzyme, cleaving after lysine (K) and arginine (R) except when followed by proline (P) and up to one missed cleavage was considered. Mass tolerance was limited to 10 ppm at the precursor and 0.02 Da at the fragment level. Carbamidomethylation of cysteine (C) was set as fixed modification and oxidation of methionine (M) as well as loss of methionine at the protein N-terminus were set as variable modifications. Identified spectra were rescored using Percolator [15] as implemented in PD and filtered for 1% FDR at the peptide spectrum match and peptide level. Based on this set of common search parameters several database search engines were evaluated for their performance of identifying spectra from low sample amounts, namely MS Amanda [16], Sequest HT [17] and Mascot 2.2 [18]. MS Amanda and Sequest HT were evaluated with and without their "second-search" feature activated, allowing for the identification of multiple peptides from single mixed MS/MS spectra. Furthermore, the novel INFERYS rescoring [19] based on fragmentation pattern prediction was evaluated. Lastly, also the performance of SpectroMine was evaluated, but here without employing Percolator for rescoring as SpectroMine runs as a standalone program and not within the PD suite.

RESULTS & DISCUSSION

Suppression of Peptide Adsorption to Autosampler Vial Surfaces

In order to ensure minimal sample losses due to adsorption of peptide material to sample vial surfaces and enable reliable processing of protein digest samples at concentrations ≤ 10 ng/µL, we first optimized sample dilution and storage conditions. The positive effects of adding trace amounts of PEG to the sample solvent on sample stability have already been described previously and were evaluated in this study for HeLa tryptic digest samples at a concentration of 250 pg/µL[20]. Aliquots of 100 ng/µL were diluted to 250 pg/µL in sample solvent with 0.001% (w/v) and without PEG and samples were analyzed during a period of 24h after the actual dilution. LC-MS/MS analyses performed at an interval of 4h clearly indicate the positive effect on peptide abundance when using 0.001% PEG as an additive into the sample injection solution (Supplementary Information figure 3). Whereas little or no difference in peptide abundance is observed immediately after sample dilution (abundance ratio PEG/FA close to 1 over the entire elution window), a significant increase in the most hydrophobic portion of the elution window is observed as from 4h after initial sample dilution (abundance ratio PEG/FA increases to 2-3 in the elution window from 35 to 45 min). In line with the earlier study on this topic[20], these results suggest that more hydrophobic peptides are less likely to adsorb to autosampler vial surfaces when trace amounts of PEG are added.

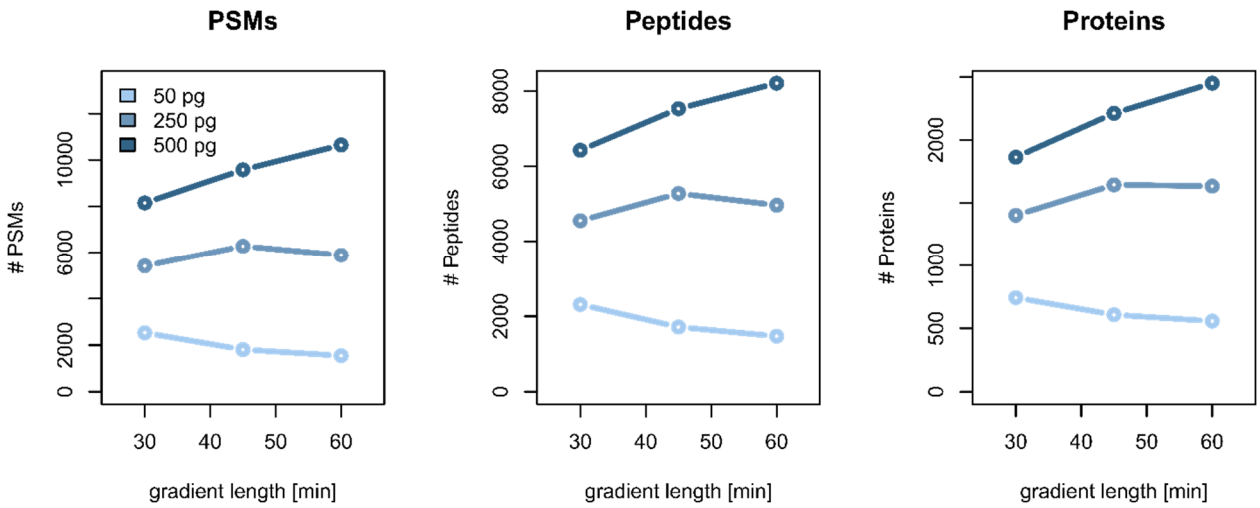

Figure 1. Effect of solvent gradient length on identifications (PSM, Peptide and Protein level). 50, 250 and 500 pg of HeLa tryptic digest was separated using a non-linear solvent gradient of 30, 45 and 60 min.

LC Solvent Gradient Optimization Towards Sub Nanogram Proteomics

Next, we evaluated the effect of solvent gradient length to ensure both maximum separation and sufficient concentration to generate clean MS/MS spectra and thereby achieve maximum number of identifications. Even though higher separation resolution is typically achieved by extending the LC solvent gradient, the relative concentration of eluting peptides decreases as a function gradient length [21–23]. As a result, the concentration of low abundant peptides will drop below the limits of detection at a certain point and further increase in peak capacity will not yield more or even less identifications. The optimal gradient length largely depends on the amount of peptide material injected, which becomes clear when comparing the number of identifications that could be obtained for HeLa peptides samples with a concentration of 50, 250 and 500 pg/µL (Figure 1). Even though peak capacity increases from 418 to 638 by extending the gradient length from 30 to 60 min (Supplementary information figure 2), no increase in identifications is observed when 50 pg of HeLa cell digest was injected. However, using longer solvent gradients does have an impact for higher sample loads, resulting in a maximum number of identified protein groups (2449 for 500 pg HeLa cell digest) with a 60 min solvent gradient. Therefore, the 60 min gradient LC method was used to explore the proteome coverage that could be achieved for sample loads up to 5 ng of HeLa tryptic peptides.

Optimized Search Strategies Enhance Proteome Coverage

As different search strategies might also impact the sensitivity achieved in low input experiments, we evaluated various data processing workflows for their ability to identify spectra from low sample amounts. The benchmark results (Figure 2) show a clear benefit for modern database search algorithms and their capability to identify multiple peptides from single mixed fragment spectra (“second search”). Strikingly, this performance improvement increases with concentration (+4.5% peptides at 250 pg to +24% more peptides at 5 ng for Sequest HT) probably due to more frequent occurrence of mixed spectra and wider isolation window for MS2 fragmentation (2 Da). Interestingly, second search leads to decreased performance at 50 pg. An opposite effect is observed for considering predicted fragmentation patterns (INFERYS), where lower concentrations show higher benefit (+22% at 50 pg to +4% at 5 ng, also Sequest HT). At 50 pg, Mascot did not produce enough identifications to allow Percolator separation between targets and

decoys and therefore no high confident identifications could be deduced. MS Amanda and SpectroMine can achieve comparable performance at sample loads exceeding 1 ng, but at sample loads below 1 ng, Sequest HT together with INFERYS results in the highest proteome coverage. Consequently, we continued further analysis using this Sequest HT / INFERYS workflow and coupled it to IMP-apQuant [24] for label-free quantification and evaluation of chromatographic parameters (i.e. FWHM).

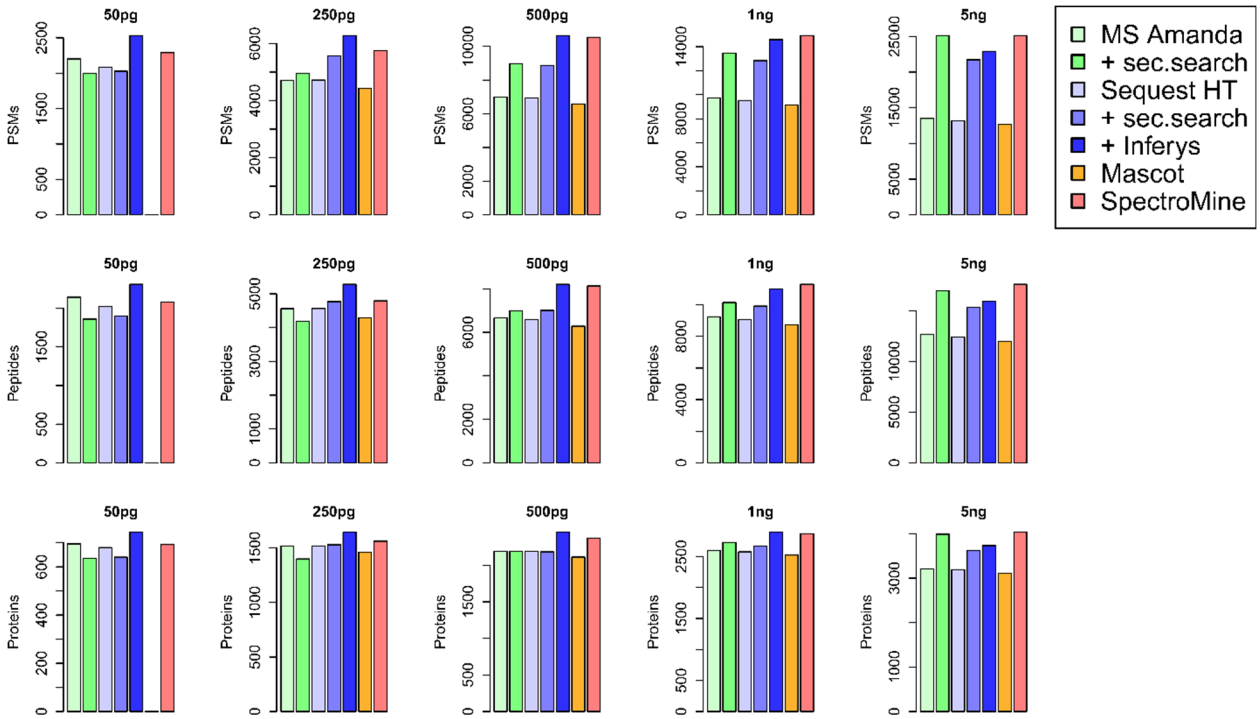

Figure 2. Impact of different search strategies on the sensitivity achieved in low input experiments. MS/MS spectra from raw data acquired at various concentrations of HeLa tryptic digest were analyzed in Proteome Discoverer (PD) to evaluate MS Amanda, Sequest HT, Mascot 2.2 and SpectroMine (not in PD). MS Amanda and Sequest HT were evaluated with and without their "second-search" feature activated, INFERYS rescoring based on fragmentation pattern prediction was evaluated with Sequest HT.

Deep and Robust Proteome Profiling of Low Sample Amounts

When implementing the Sequest HT / INFERYS workflow for a dilution series of HeLa tryptic digest (0.25, 0.5, 1, 2.5 and 5 ng), we demonstrate identification and quantification of 1486 protein groups on average from just 250 pg (Figure 3a), which approaches the amount of protein expressed in a single cell [25]. Overall, these results clearly demonstrate the progress achieved due to the improved chromatographic setup and combination of Orbitrap MS with FAIMS, as now we can identify 2888 proteins from 1 ng of peptides, whereas in the previous contribution, 2436 proteins could be identified from 10 ng in a 1 hr gradient [14] (Figure 3b). To assess proteome coverage achieved from low input samples, we overlayed peptide abundance in a 5 ng/µL sample with their detection in lower sample amounts (50, 250, 500 pg/µL). Low sample amounts allow for the detection of abundant peptides, whereas less abundant peptide species cannot be detected from low sample amounts (Figure 3c). This is expected and scales with the amount injected. Comparing the ten-percentile apQuant Area of peptides at a concentration of 5 ng/µL for peptides detected at low concentration results in 1.3e6 for peptides detected at 500 pg and increases to 1.7e7 for peptides detected in 50 pg corresponding to a factor of 12.6. So, detection of a peptide

in a 10-fold lower concentration here requires 12.6-fold higher peptide abundance. It is expected that only abundant peptides can be detected from lower concentrations, especially for homogenous mixtures of cell digest derived from larger cell populations. We hypothesize that single cell samples could enable detection of less abundant peptides, as bulk analysis might mask some higher abundant peptides originating from only a subpopulation of cells.

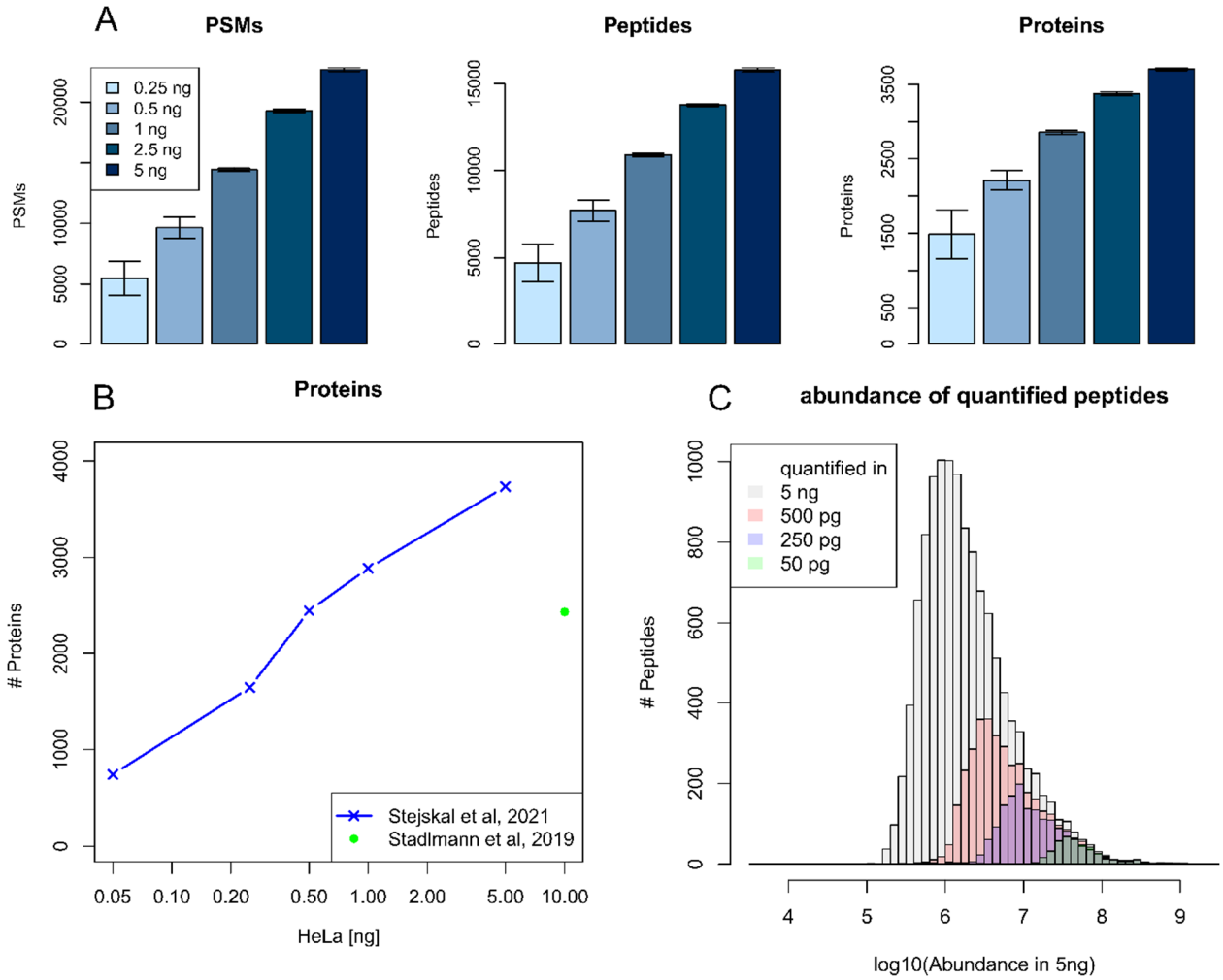

Figure 3. A) PSM, peptide and protein identifications obtained for a dilution series of HeLa tryptic digest (5 to 0.25 ng injected), Sequest HT with INFERYS rescoring was used as processing workflow in PD 2.5. (average values of technical quadruplicates, n=4) B) The amount of proteins identified as a function of the sample load (ng). Current data is compared to results obtained in 2019 by our group (14). C) Comparison of the abundance of quantified peptides obtained for a 5 ng/µL sample to their detection in lower sample amounts (50, 250, 500 pg/µL).

CONCLUSION

The combination of optimized sample dilution and storage conditions, a dedicated second generation limited sample micro pillar array column, Orbitrap Exploris™ 480 mass spectrometer, FAIMS Pro interface and the latest database search algorithms provides a very promising solution for the analysis of low input protein digest samples. More than a 10-fold increase in detection sensitivity was achieved as compared to a previous contribution, where we reported on identification of 2436 proteins from 10 ng of HeLa tryptic digest. By identifying 1486 and 2210

proteins from as little as 250 and 500 pg of HeLa tryptic digest respectively, we demonstrate the potential of the current setup for integration in limited sample and single cell proteomics workflows.

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Spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE [26] partner repository with the dataset identifier PXD024017.

Username: reviewer_pxd024017@ebi.ac.uk

Password: uaC21Wg2

SUPPLEMENTARY INFORMATION

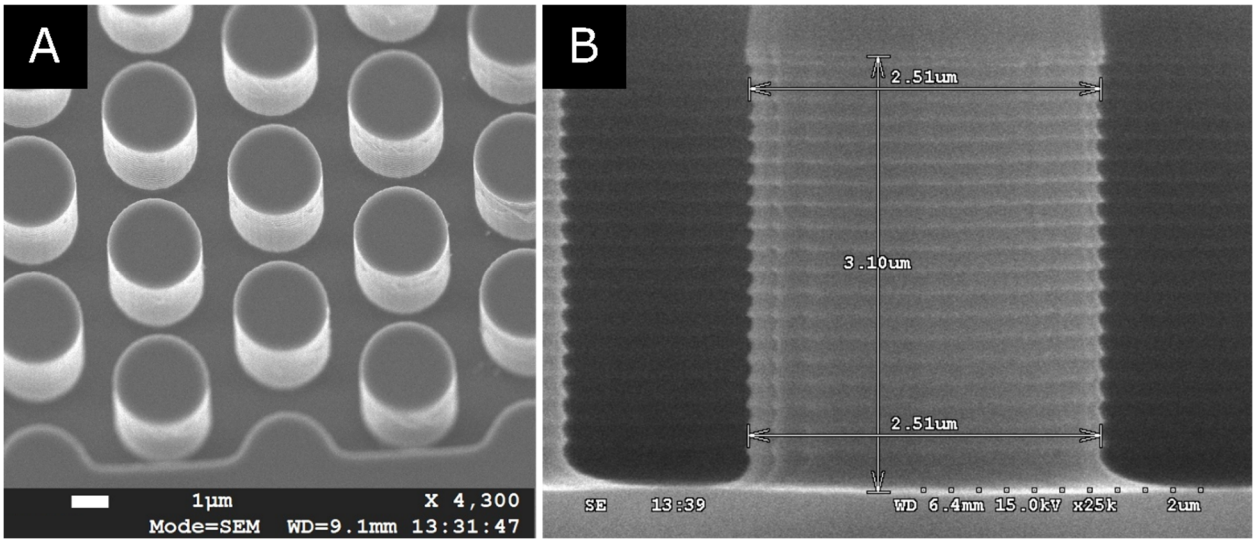


Figure SI1. A) Scanning Electron Microscopy (SEM) image showing a birds eye view of the second generation micro pillar array stationary phase. B) SEM image showing a transverse section of the micro pillar array column, zoom in on a single 2.5 µm diameter non-porous micro pillar.

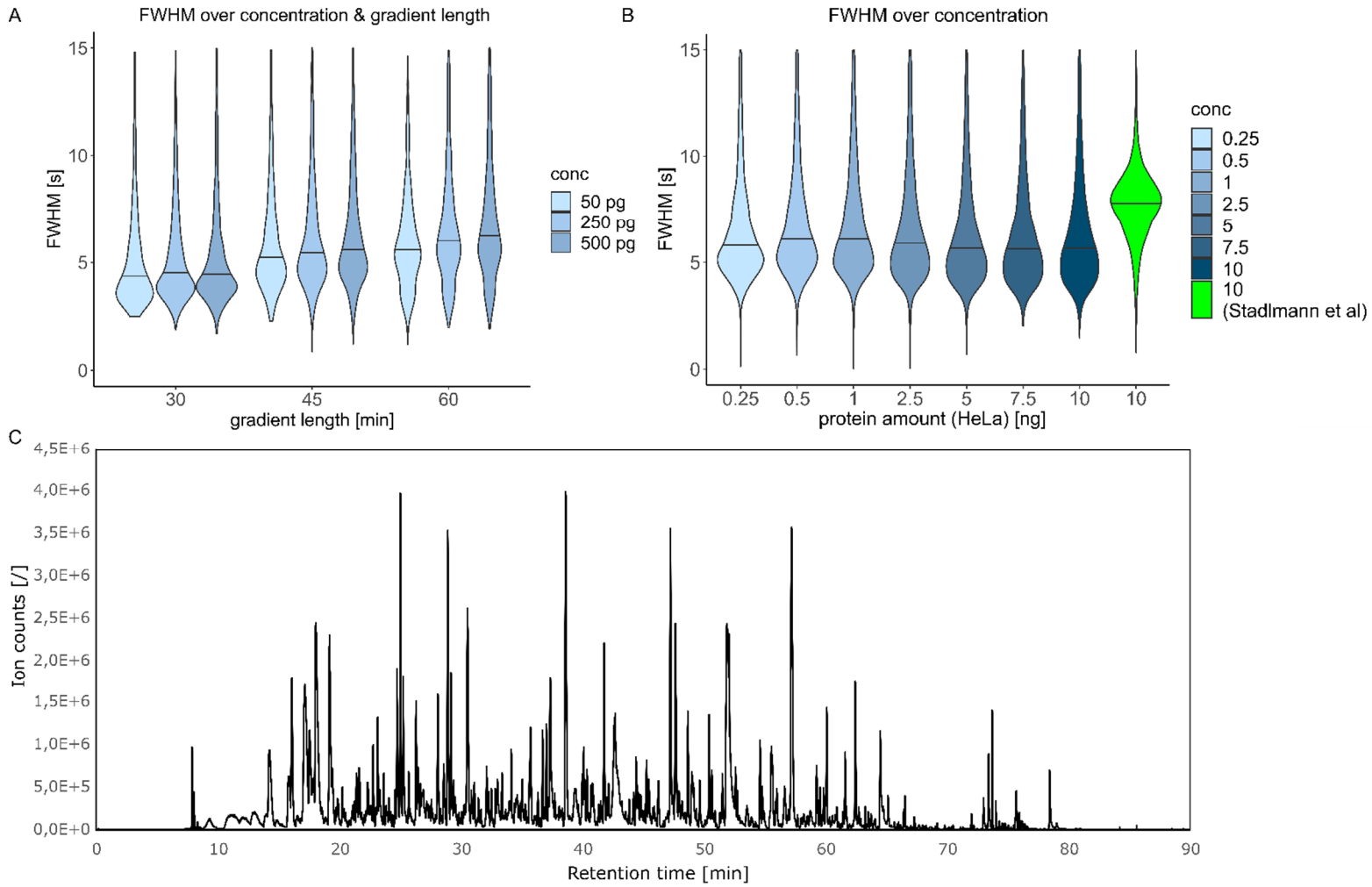


Figure SI2. A) Median FWHM values obtained for all identified peptides as a function of the sample load (50, 250 and 500 pg HeLa tryptic digest) and gradient length (30, 45 and 60 min). B) Median FWHM values obtained for all identified peptides as a function of the sample load (0.25, 0.5, 1, 2.5, 5, 7.5, 10 ng HeLa tryptic digest). Current data is compared to results obtained in 2019 with a first generation superficially porous micro pillar array column (14). C) MS basepeak chromatogram obtained for the separation of 250 pg HeLa tryptic digest using a 60 min non-linear gradient at 250 nL/min

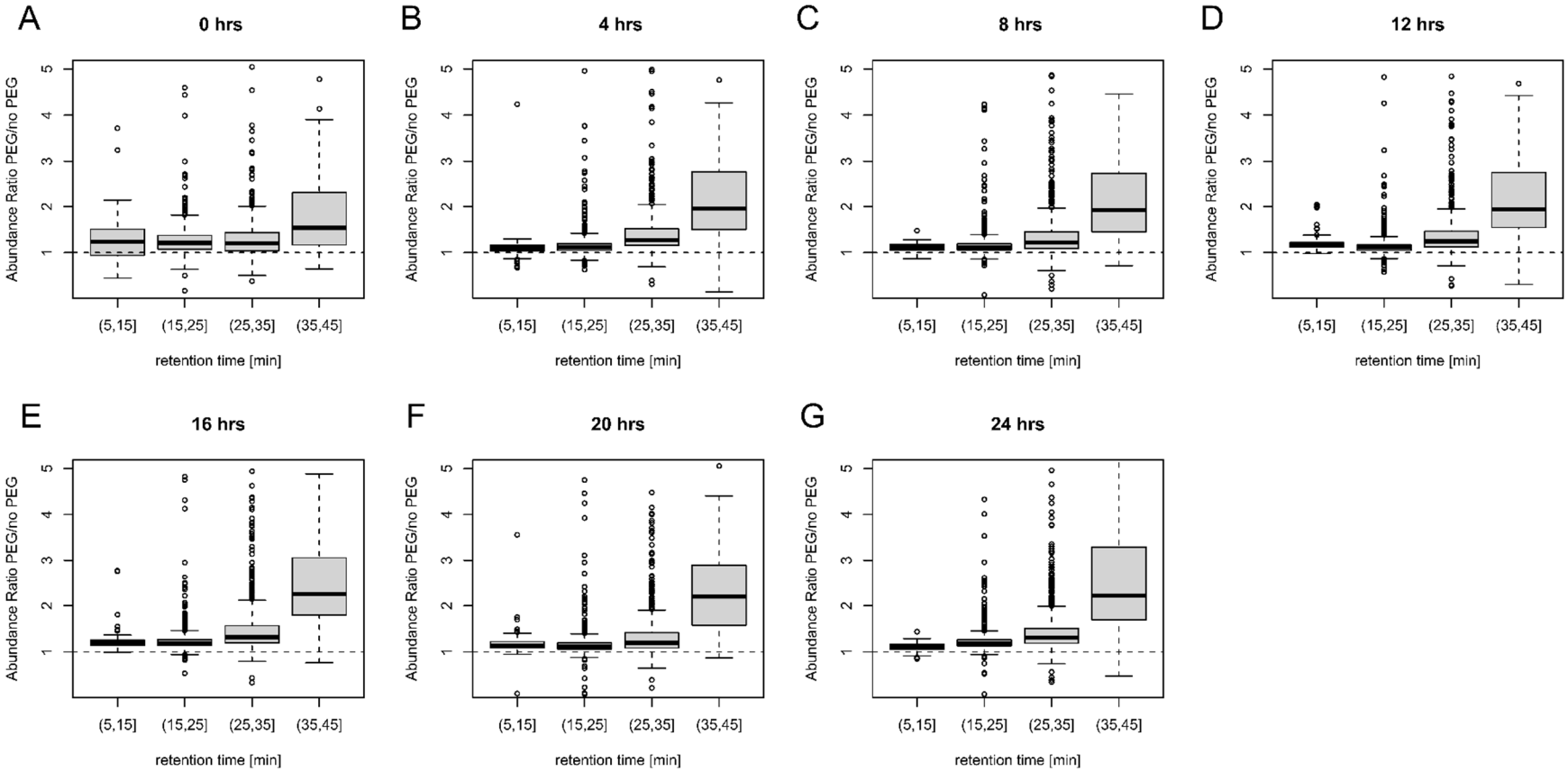


Figure SI3. A-G) Abundance ratio obtained on the peptide level for samples where PEG (0.001% w/v) and no PEG was added to the sample solvent (0.1% FA). Abundance ratio has been plotted for 4 distinct elution windows (5-15,15-25, 25-35 and 35-45 min) representative for the increasing hydrophobicity of peptides. Results obtained over a period of 24h after initial sample dilution are shown at an interval of 4h. F) Total amount of PSMs obtained for samples where either PEG (0.001% w/v) or no PEG was added. Results obtained over a period of 24h after initial sample dilution are shown at an interval of 4h.

Limited Sample Micro Pillar Array Column

The nature of the pillar array fabrication process allows precise control over the stationary phase morphology and can therefore be finetuned towards certain applications [1,2]. In an attempt to further tune the pillar array based stationary phase towards optimal compatibility with very low sample amounts, three important adaptations have been made to the column design. Advanced deep UV lithography has been used to reduce the pillar dimensions by a factor of 2, yielding an array of 2.5 µm diameter silicon pillars positioned at a distance of 1.25 µm (supplementary information figure 1). By reducing the distance that molecules have to travel to interact with the stationary phase, the effect of resistance to mass transfer on peak dispersion is reduced is and more efficient chromatography can be achieved [3]. This ultimately results in an increased signal to noise ratio when entering the MS. By using non-porous instead of superficially porous pillars, an additional gain in sensitivity can be achieved through a complementary effect on peak dispersion. Interaction with the stationary phase is restricted to the outer pillar shell instead of the mesoporous mantle (or the entire mesoporous matrix in the case of fully porous particles). This has a beneficial effect on separation performance separation as long as the interaction surface of the pillars is not fully saturated (and sample overloading starts having a detrimental effect on separation performance). And finally, further reduction of the channel cross section by a factor of 2 ensures optimal peptide elution at flow rates between 100 and 250 nL/min, exploiting the increased electrospray ionization efficiencies associated with low end nanoflow LC [4].

Chromatographic Metrics

Integration of the IMP-apQuant node [5] into the PD processing workflow enables convenient and automated evaluation of chromatographic parameters (i.e. FWHM, kurtosis, skewness) for bottom-up MS/MS analyses of complex protein digest samples. The FWHM distribution obtained for different sample loads (50, 250 and 500 ng HeLa tryptic digest) and different gradient lengths have been plotted in supplementary information figure 2a and give a clear representation of the chromatographic performance that can be achieved with the novel micro pillar array column. Based on the median FWHM value that was observed for a 30, 45 and 60 min solvent gradient, peak capacity values ranging from 400 for the shortest to 640 for the longest gradient could be obtained. When comparing these data to the results we previously published using a micro pillar array column with superficially porous pillars and pillar dimensions that are twice the size of those of the current column, a significant reduction of the FWHM values (from 7.94 to 5.64 s; 29% reduction) is be observed. These values are not in full agreement with what was published in 2019, because improved FWHM calculation in apQuant is used. Whereas the previous version only measured time between data points that are above half maximum, the current version uses linear interpolation on both sides which is more accurate. A net gain in peak capacity of 41% (from 543 to 638) is demonstrated, which can mainly be attributed to the reduction of the pillar diameter and interpillar spacing (supplementary information figure 2b). Further downscaling of the channel cross section and the absence of a meso porous layer has an effect on the mass loadability of the column (reduction by a factor of approximately 30). Excellent chromatography with no signs of excessive column overloading can however be achieved for samples with a total protein content up to 10 ng. This is illustrated in supplementary information figure 2b, where FWHM distributions obtained for a HeLa tryptic digest dilution series from 10 down to 0.25 ng are compared. Further downscaling of the column cross section also has a positive effect on the elution profiles that can be achieved when working at low nano LC flow rates. With a total column volume (including connection lines) of 1.25 µL (instead of 3 µL for the micro pillar array column used in the previous

publication), more efficient use of LC-MS/MS instrument time can be obtained (supplementary information figure 2c). When performing direct injection (1µL sample loop, volume is added to the total column volume) and operating the column at a flow rate of 250 nL/min, peptides start eluting as from 11.5 min after sample injection.

Supplementary Information References

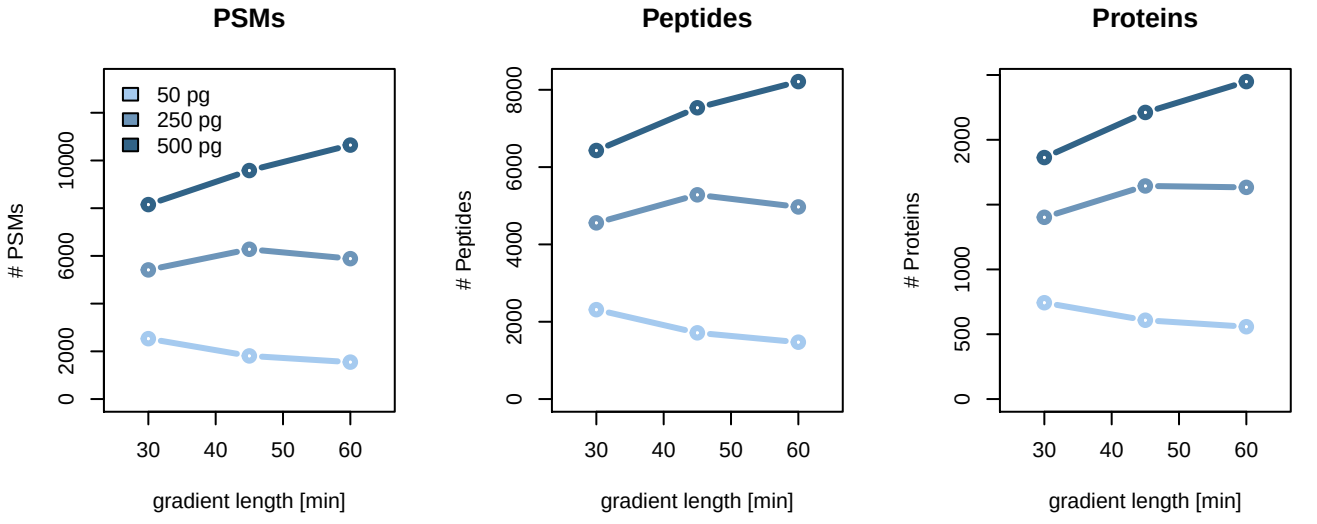
(1) De Malsche, W.; Gardeniers, H.; Desmet, G. Experimental Study of Porous Silicon Shell Pillars under Retentive Conditions. *Anal. Chem.* **2008**, *80* (14), 5391–5400. https://doi.org/10.1021/ac800424q.

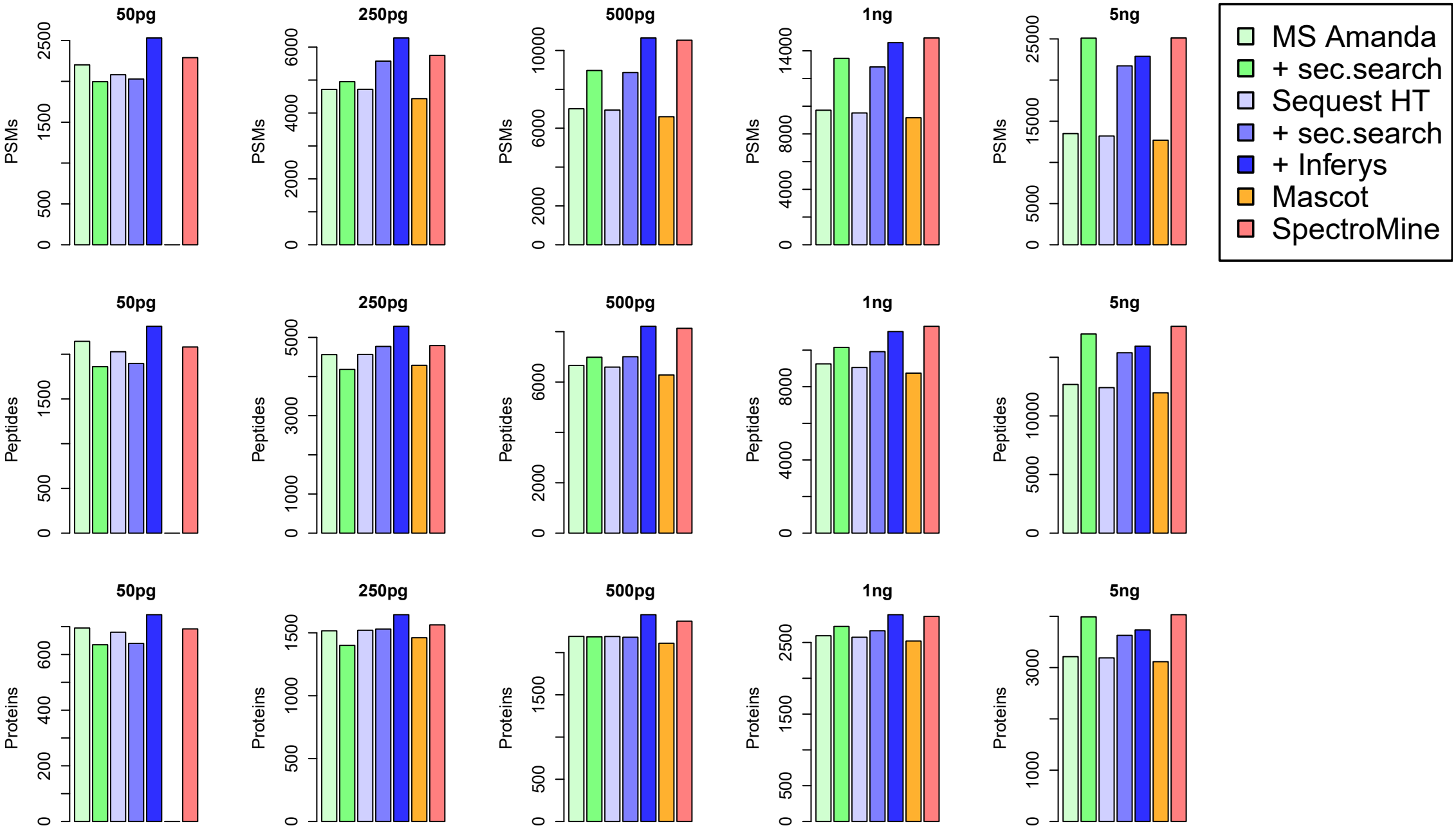
(2) Desmet, G.; Op de Beeck, J.; Van Raemdonck, G.; Van Mol, K.; Claerebout, B.; Van Landuyt, N.; Jacobs, P. Separation Efficiency Kinetics of Capillary Flow Micro-Pillar Array Columns for Liquid Chromatography. *J. Chromatogr. A* **2020**, *1626*, 461279. https://doi.org/10.1016/j.chroma.2020.461279.

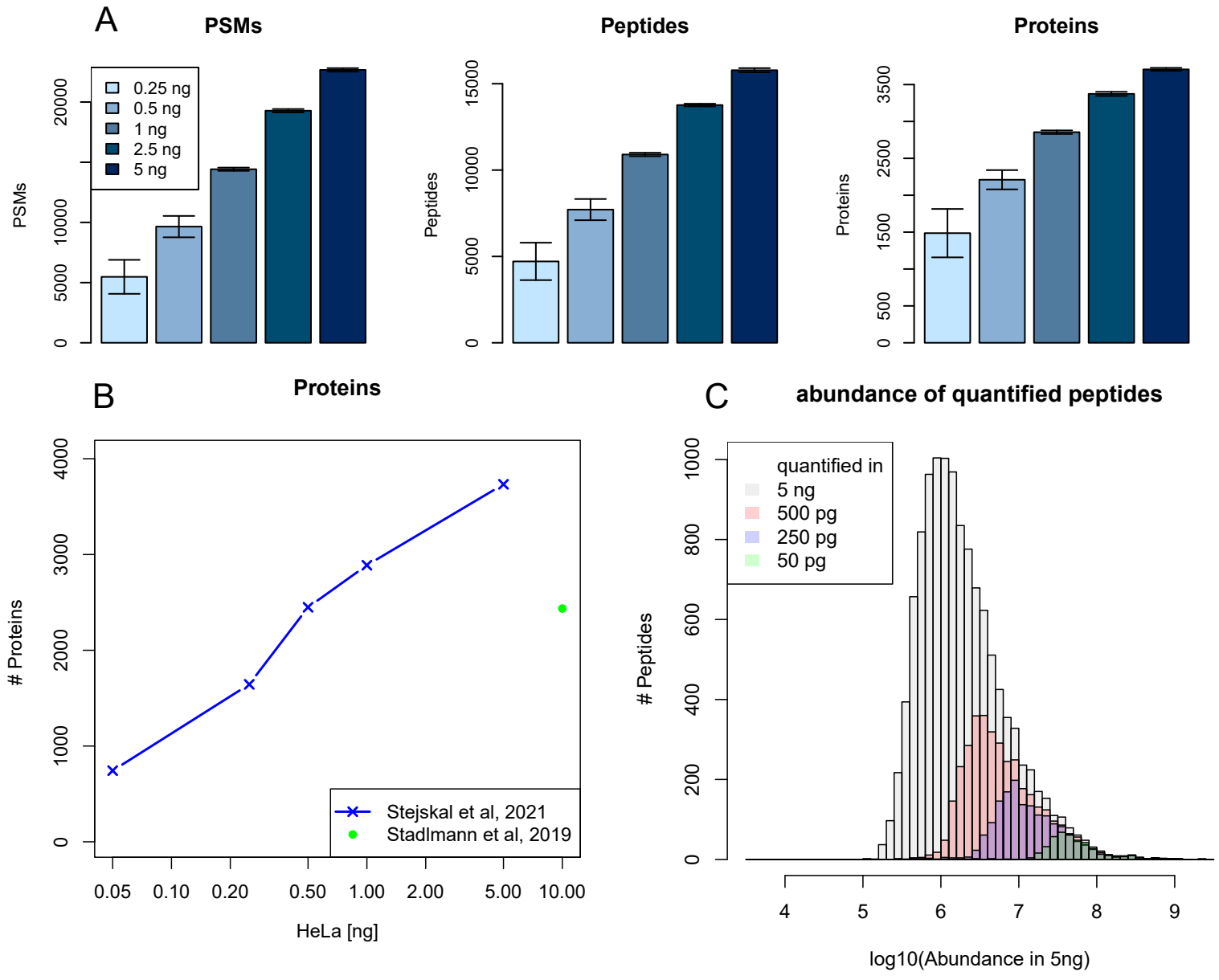
(3) Gritti, F.; Guiochon, G. Mass Transfer Kinetics, Band Broadening and Column Efficiency. *J. Chromatogr. A* **2012**, *1221*, 2–40. https://doi.org/10.1016/j.chroma.2011.04.058.

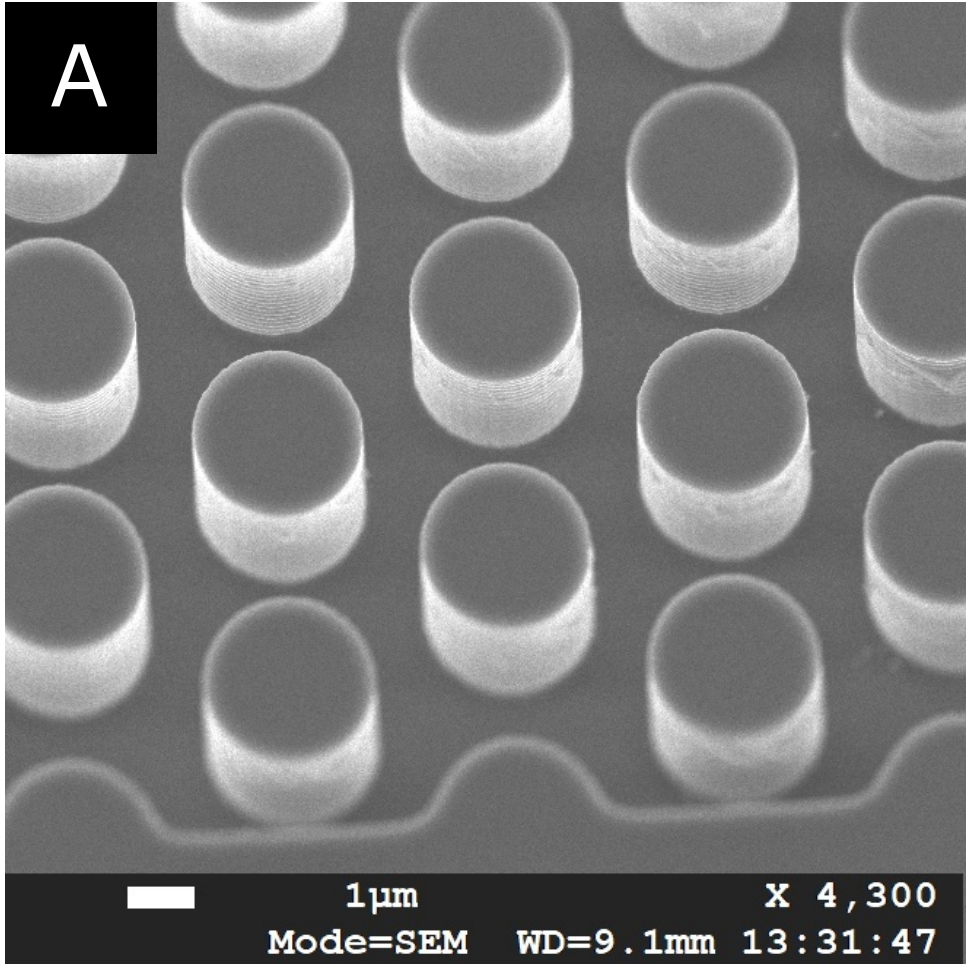
(4) El-Faramawy, A.; Siu, K. W. M.; Thomson, B. A. Efficiency of Nano-Electrospray Ionization. *J. Am. Soc. Mass Spectrom.* **2005**, *16* (10), 1702–1707. https://doi.org/10.1016/j.jasms.2005.06.011.

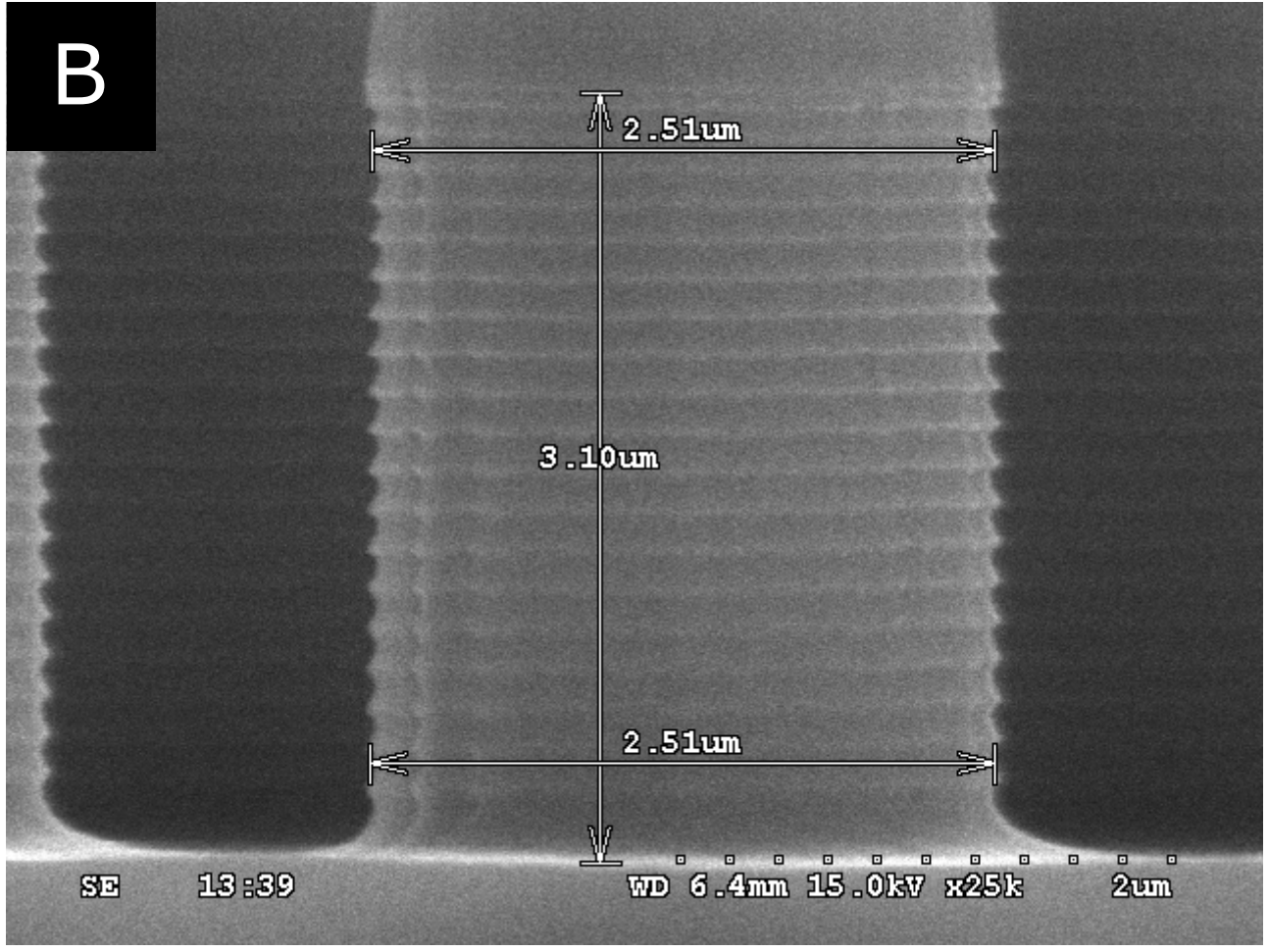
(5) Doblmann, J.; Dusberger, F.; Imre, R.; Hudecz, O.; Stanek, F.; Mechtler, K.; Dürnberger, G. ApQuant: Accurate Label-Free Quantification by Quality Filtering. *J. Proteome Res.* **2019**, *18* (1), 535–541. https://doi.org/10.1021/acs.jproteome.8b00113.

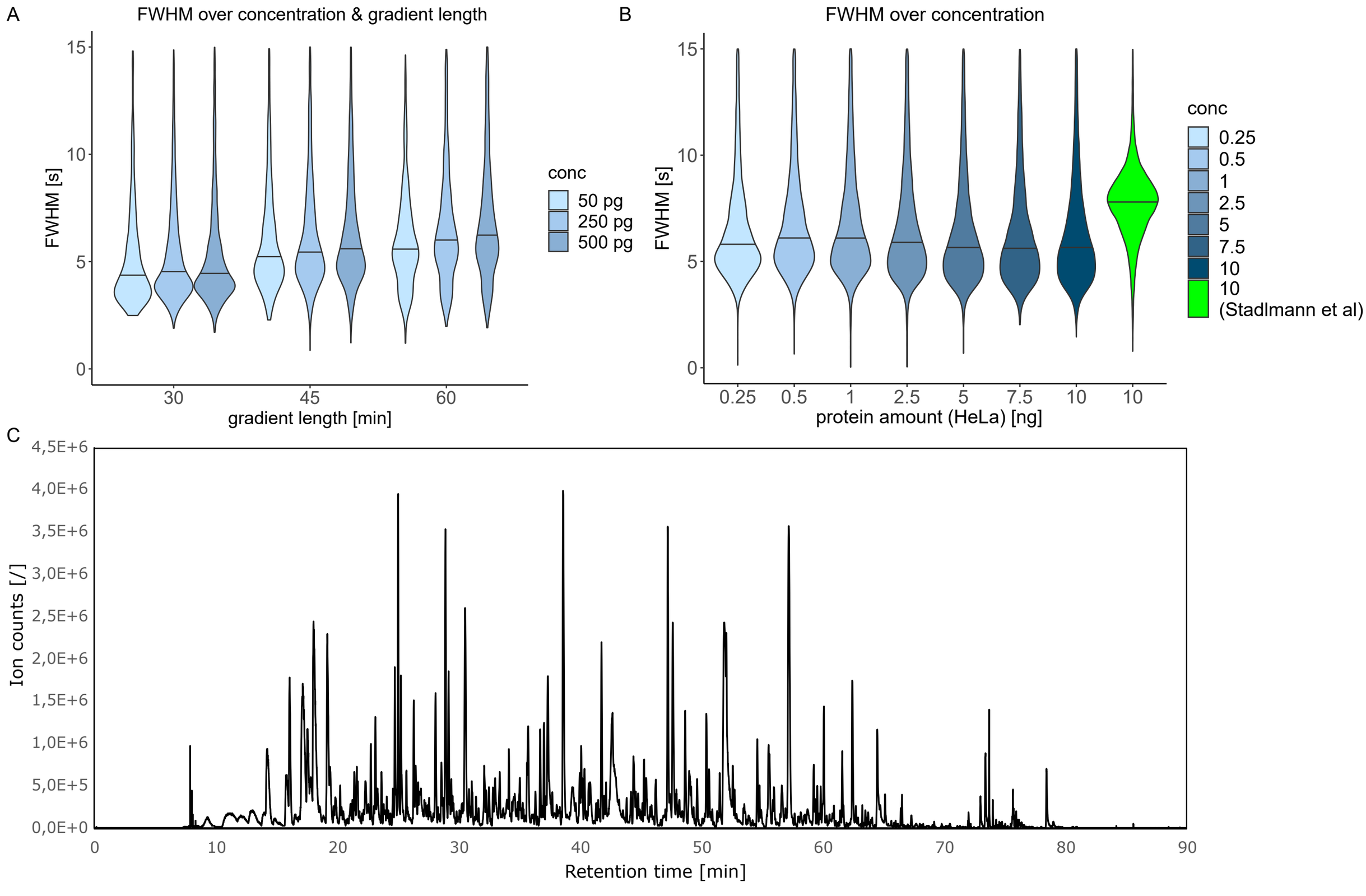

PSMs
Peptides
Proteins
50 pg
250 pg
500 pg
PSMs
Peptides
Proteins
0
2000
6000
10000
4000
8000
500
1000
30
40
50
60
gradient length [min]

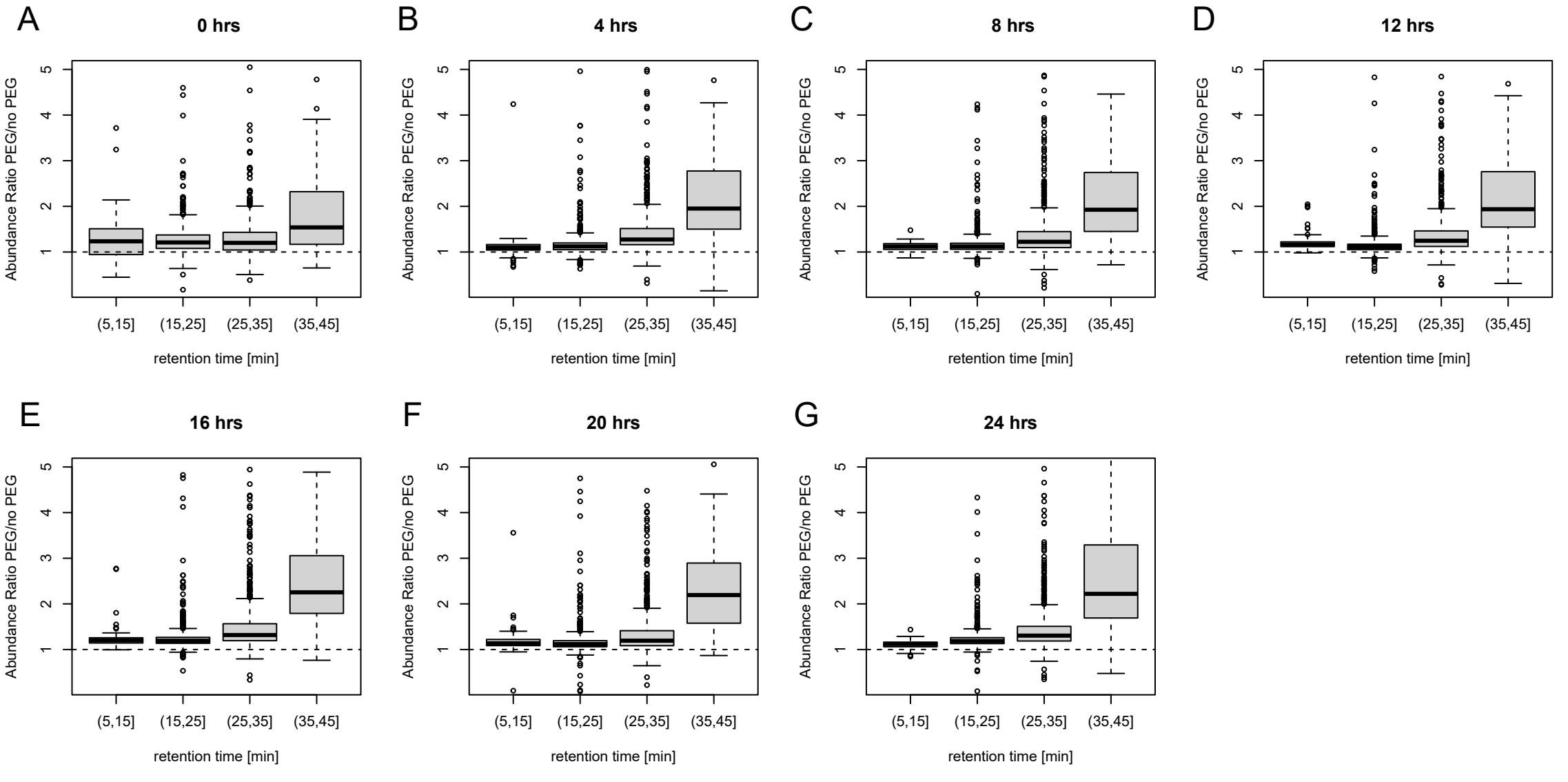

50pg
250pg
500pg
1ng
5ng
PSMs
Peptides
Proteins
MS Amanda
+ sec.search
Sequest HT
+ sec.search
+ Inferys
Mascot
SpectroMine


A
PSMs
Peptides
Proteins
0.25 ng
0.5 ng
1 ng
2.5 ng
5 ng
PSMs
Peptides
Proteins
B
Proteins
Proteins
HeLa [ng]
0.05
0.10
0.20
0.50
1.00
2.00
5.00
10.00
Stejskal et al, 2021
Stadlmann et al, 2019
C
abundance of quantified peptides
quantified in
5 ng
500 pg
250 pg
50 pg
# Peptides
log10(Abundance in 5ng)


A
1µm
X 4,300
Mode=SEM WD=9.1mm 13:31:47


B
2.51um
3.10um
2.51um
SE 13:39
WD 6.4mm 15.0kV x25k
2um


A
FWHM over concentration & gradient length
FWHM [s]
gradient length [min]
conc
50 pg
250 pg
500 pg
B
FWHM over concentration
FWHM [s]
protein amount (HeLa) [ng]
conc
0.25
0.5
1
2.5
5
7.5
10
10
(Stadlmann et al)
C
Ion counts [/]
Retention time [min]


A
0 hrs
B
4 hrs
C
8 hrs
D
12 hrs
E
16 hrs
F
20 hrs
G
24 hrs
Abundance Ratio PEG/no PEG
1
2
3
4
5
(5,15]
(15,25]
(25,35]
(35,45]
retention time [min]