# Do we still need nanospray for exploratory proteomics?

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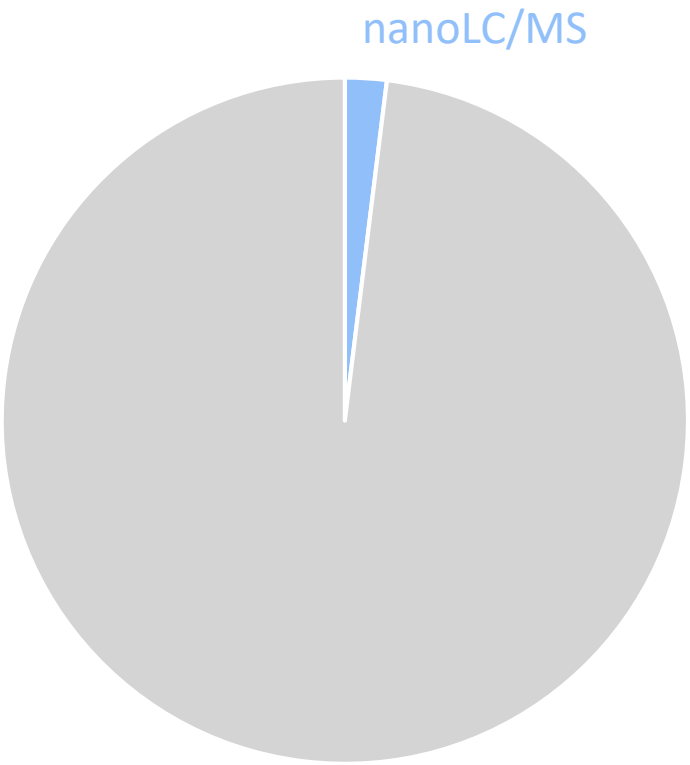
Charles University

# Background

## LC-MS installations




Cell Systems
Commentary

## Now, More Than Ever, Proteomics Needs Better Chromatography

Evgenia Shishkova,[1] Alexander S. Hebert,[2] and Joshua J. Coon[1,2,3,*]
[1]The Department of Biomolecular Chemistry
[2]The Genome Center of Wisconsin
[3]The Department of Chemistry
University of Wisconsin – Madison, Madison, WI, USA

# Methods

## Q Exactive Plus

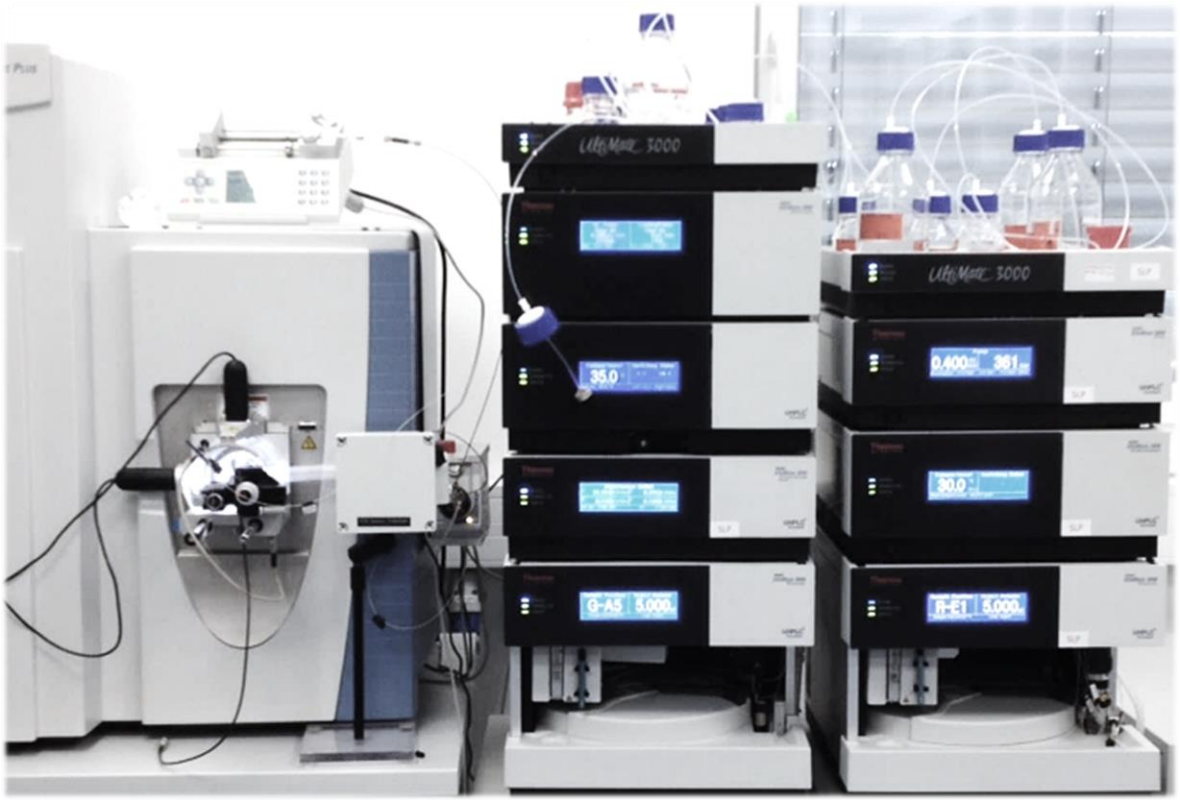
- nanoLC/nanoESI
- conv. flow LC/HESI-II

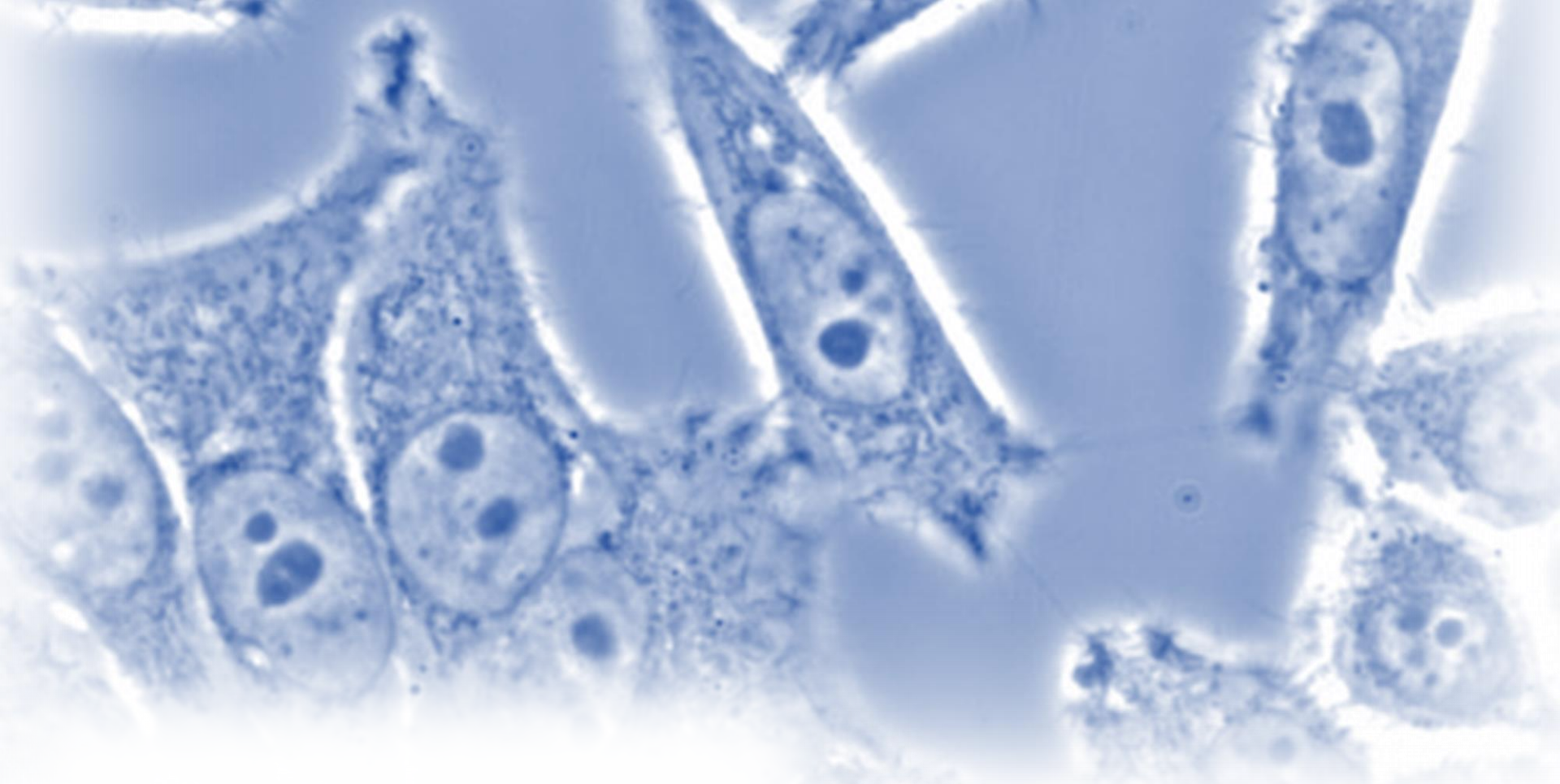
Identical sample

Identical gradient

Identical DDA method

Identical evaluation




P02769|ALBU_BOVIN Serum albumin OS=Bos taurus GN=ALB
P68082|MYG_HORSE Myoglobin OS=Equus ca

# HeLa cells

## Search parameters

Preview ™

PROTEIN METRICS

## Spectra processing

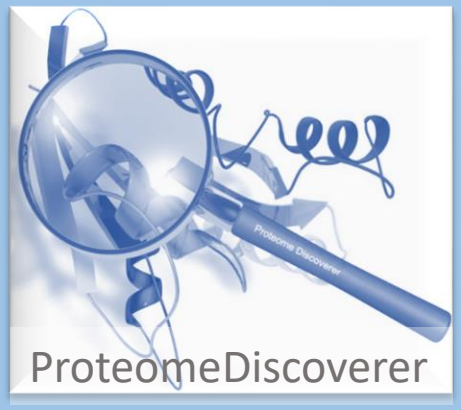
MaxQuant

Andromeda

## Main DDA search

MATRIX SCIENCE

Mascot



ProteomeDiscoverer

Percolator

Skyline

## LC peaks parameters

# DDA of HeLa rLys-C/trypsin digest

Proteins

Peptides

PSMs

MS2

PSMs/peptides

Explained MS2 [%]

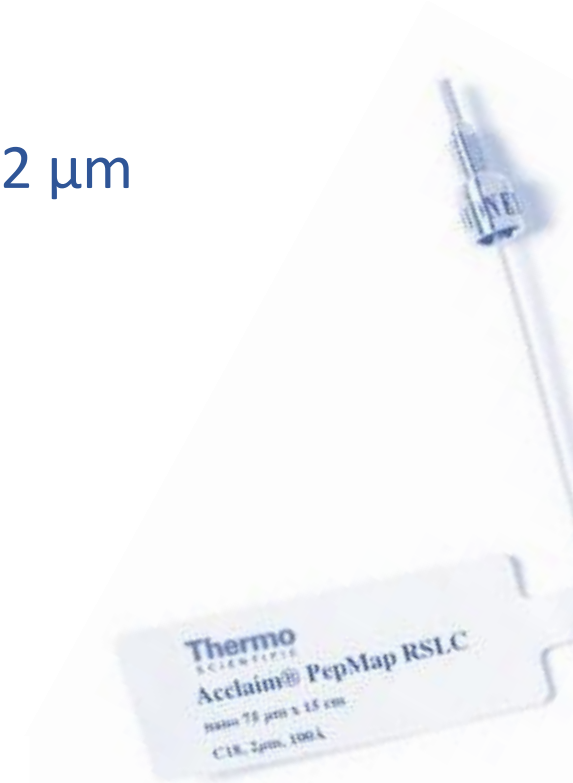
# Benchmark nanoLC

## 0.075 x 250 mm; 250 nL/min

### Acclaim™ PepMap™ C18, 100Å, 2 µm

| | Proteins | Peptides | PSMs |
|---|---|---|---|
| nano 375 ng | 2381 | 15.9k | 23.5k |

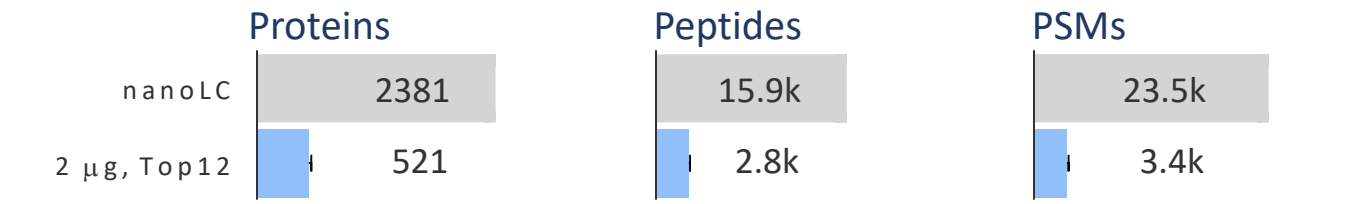
| | MS2 | PSMs/peptides | Explained MS2 [%] |
|---|---|---|---|
| nano 375 ng | 38.6k | 1.48 | 61.0 |



# Narrow-bore LC

## 2.1 x 100 mm; 300 µL/min

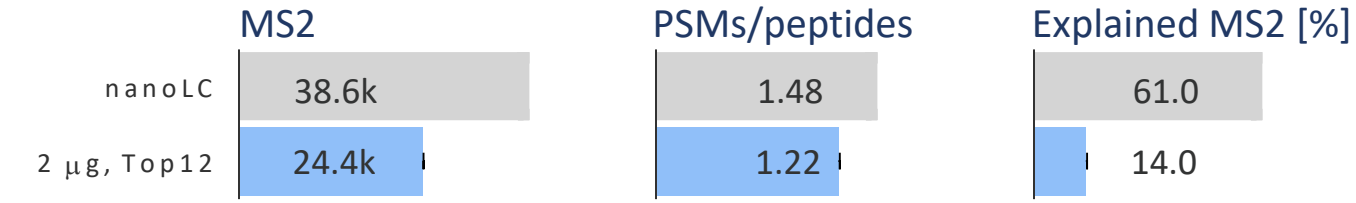
### Poroshell 120Å C18 2.7 µm









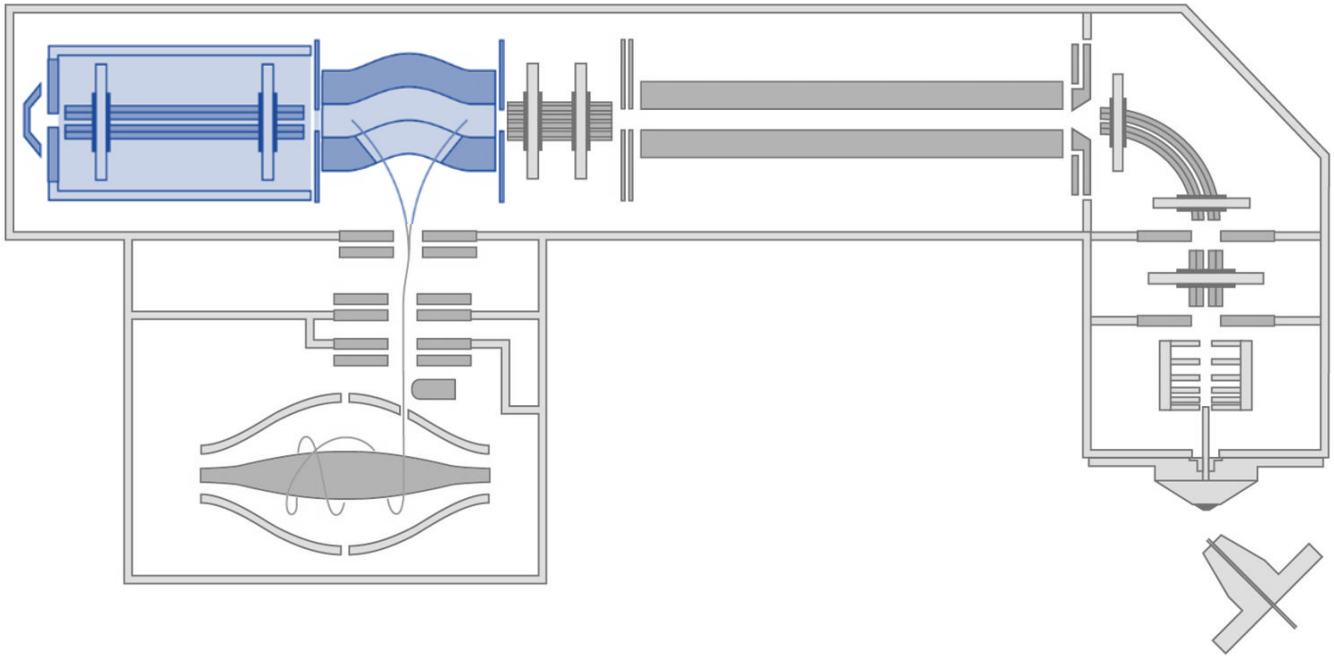










# DDA optimization

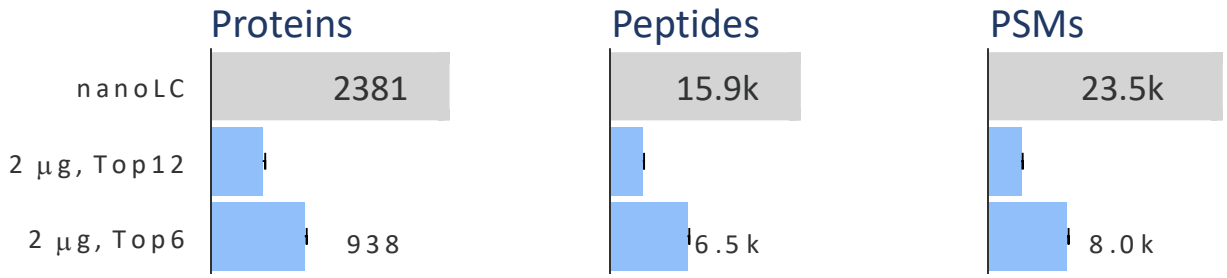


| DDA settings | Top12 | Top6 |
|---|---|---|
| Fill time [ms] | 50 | 110 |

# Narrow-bore LC

## 2.1 x 100 mm; 300 µL/min

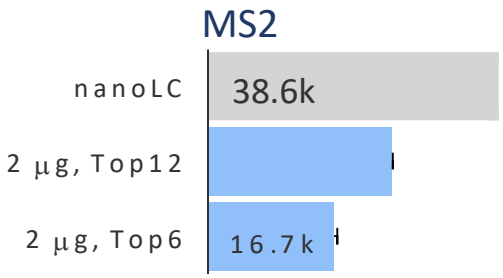
### Poroshell 120Å C18 2.7 µm

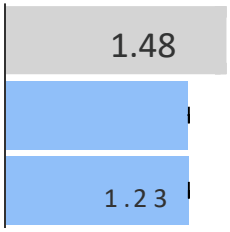


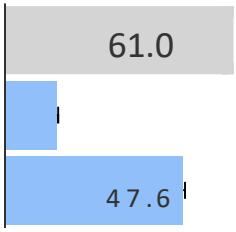






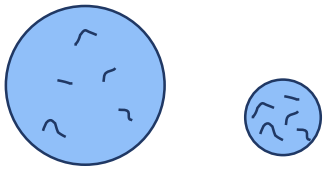




# Microbore LC

## 1.0 x 100 mm; 68 µL/min

### Poroshell 120Å C18 2.7 µm

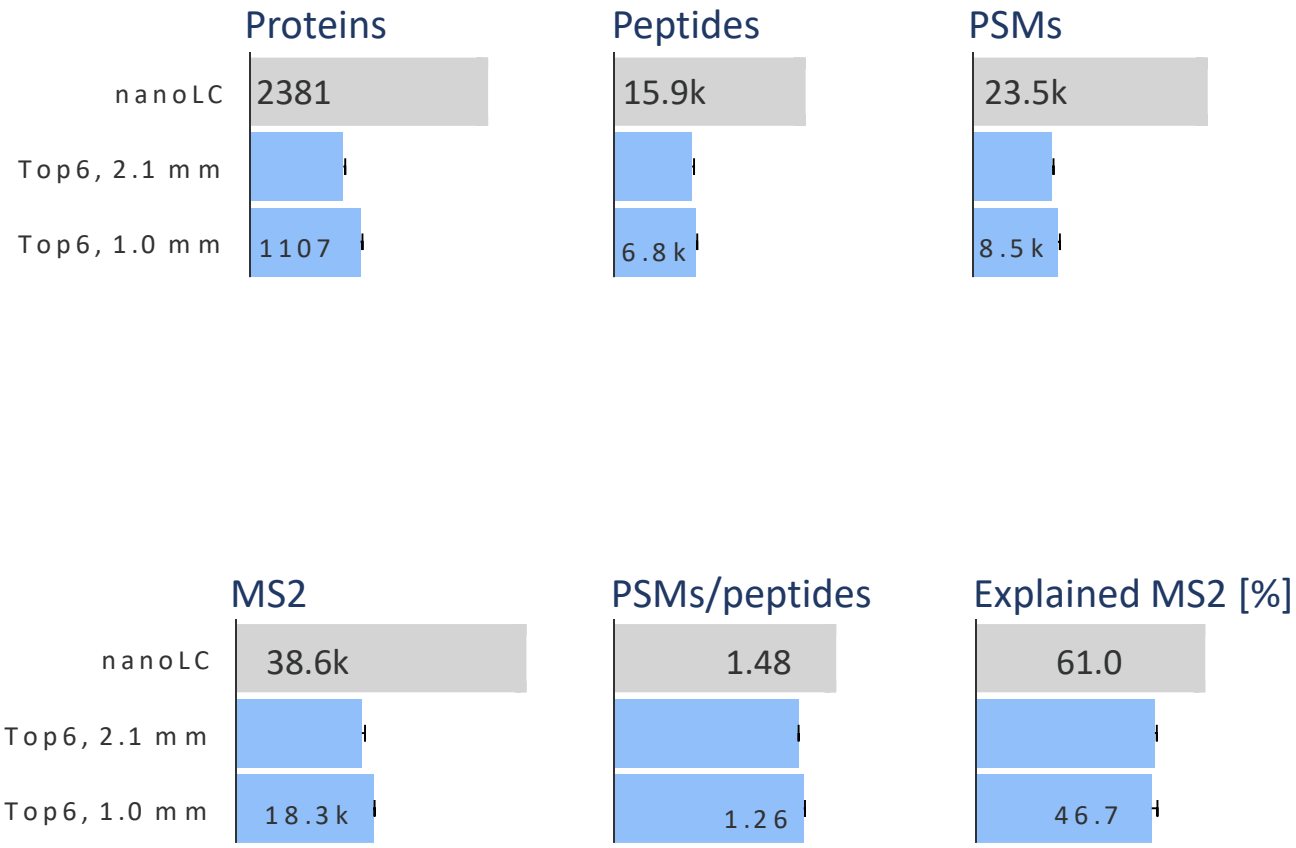


2.1 mm 1.0 mm

$$\frac{2.1^2}{1.0^2} = 4.41$$

# Microbore LC

## 1.0 x 100 mm; 68 µL/min



# Microbore LC

$$\boldsymbol{E_r = 100 \cdot \frac{\sigma_{col}^2}{\sigma_{col}^2 + \sigma_{ec}^2}}$$

$$\sigma_{inj}^2 = k_{inj} \cdot \frac{V_{inj}^2}{12}$$

$$N^* = 5.54 \left( \frac{G \cdot t_0 (1 + k_e)}{W_{0.5}} \right)^2$$

$$\sigma_{ec}^2 = \sigma_{inj}^2 + \sigma_{pre}^2 + \sigma_{post}^2 + \sigma_{det}^2 + \sigma_{other}^2$$

$$\sigma_{col}^2 = \frac{V_0^2}{N^*} \cdot (1 + k_e)^2$$

$$G = \frac{\sqrt{1 + p + \left(\frac{p^2}{3}\right)}}{1 + p}$$

$$k_e = \frac{k_0}{b \cdot k_0 + 1}$$

$$\sigma_{det}^2 = k_{cell} \cdot \frac{V_{cell}^2}{12} + \tau^2 \cdot F^2$$

$$\sigma_{tubing}^2 = \frac{\pi^2 \cdot r^4 \cdot L^2}{3 + 24 \cdot \pi \cdot L \cdot \frac{D_m}{F}}$$

$$p = \frac{2.3 \cdot k_0 \cdot b}{k_0 + 1}$$

$$k_0 = \frac{1}{b}\left(e^{b \cdot k_g} - 1\right)$$

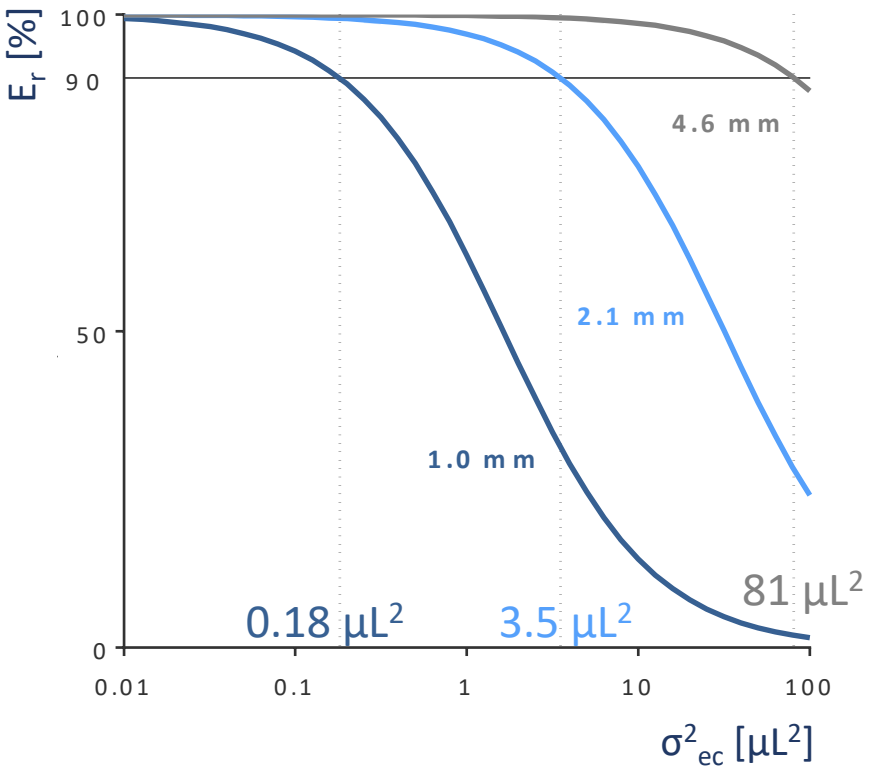
$$D_m = \frac{8.341 \cdot 10^{-8} \cdot T}{\eta \cdot \sqrt[\frac{1}{3}]{M_w}}$$

$$k_g = \frac{1}{1.15 \cdot b}$$

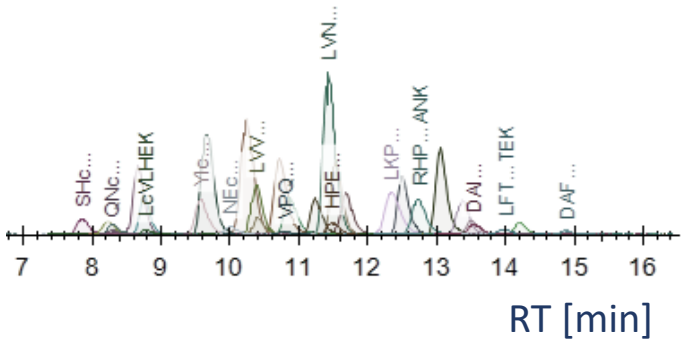
$$b = S \cdot \Delta\Phi \cdot \frac{V_0}{t_g \cdot F}$$

$$\eta = 2.414 \cdot 10^{-5} \cdot 10^{\left(\frac{247.8}{T - 140}\right)}$$

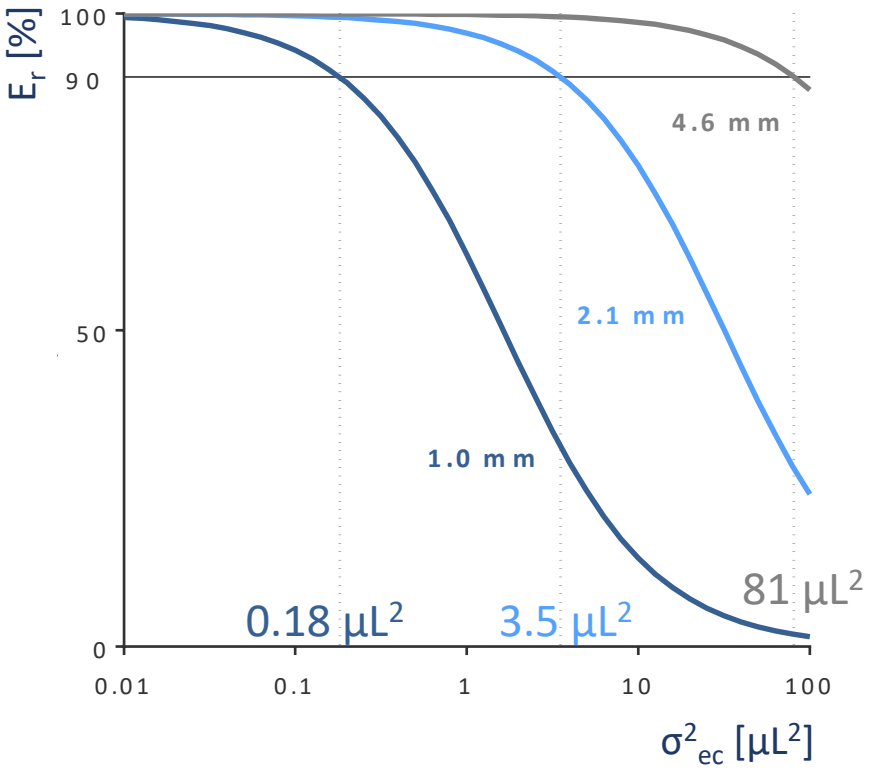
# Microbore LC

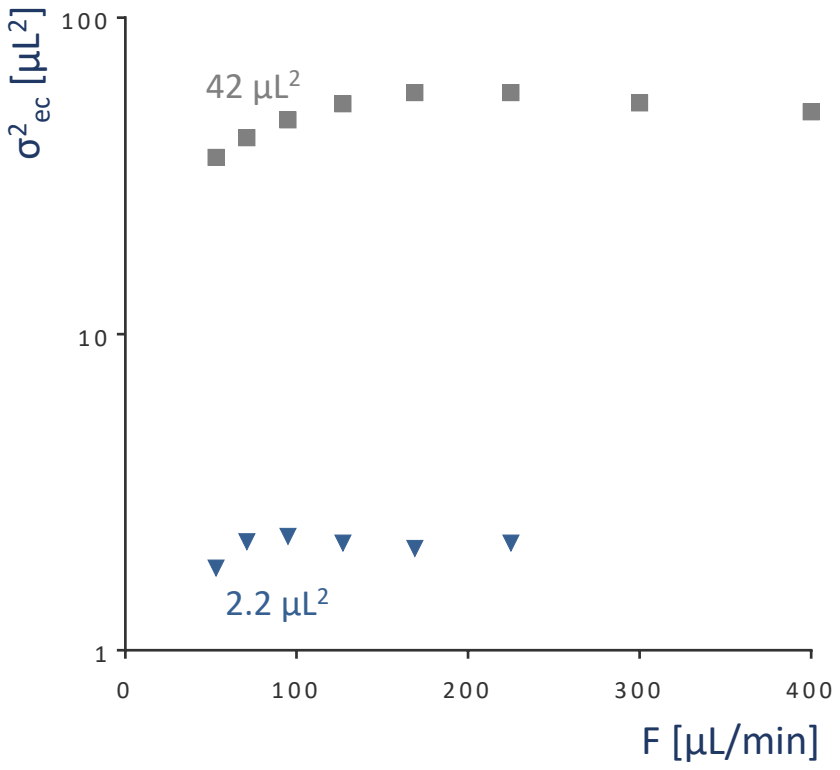



$$E_r = 100 \cdot \frac{\sigma_{col}^2}{\sigma_{col}^2 + \sigma_{ec}^2}$$

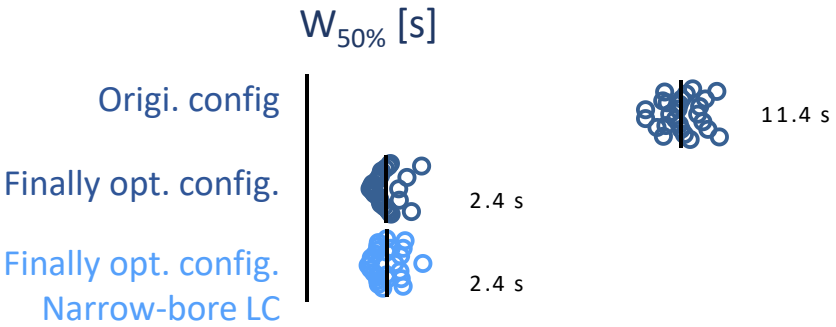


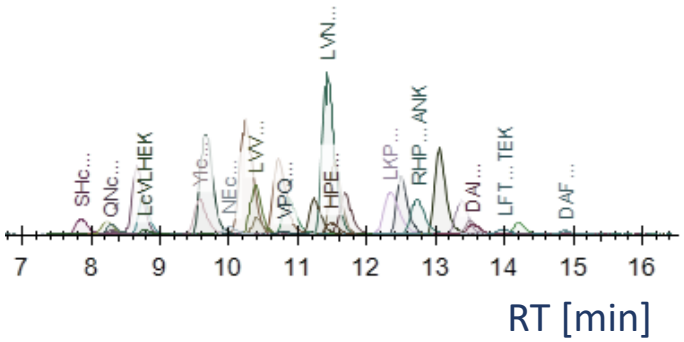
# Microbore LC

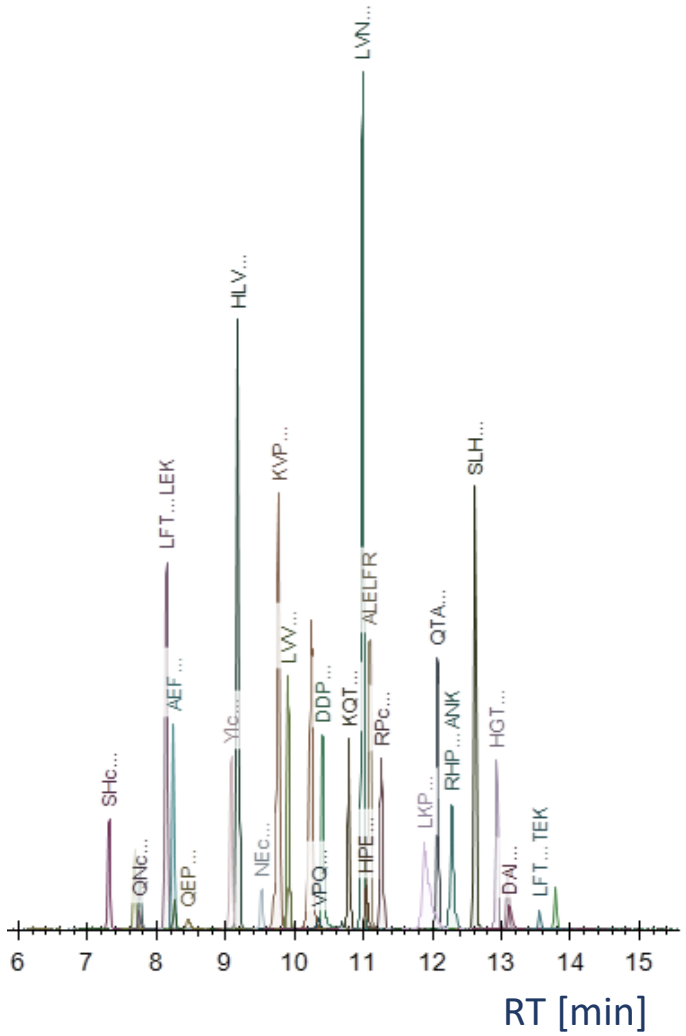



# Microbore LC

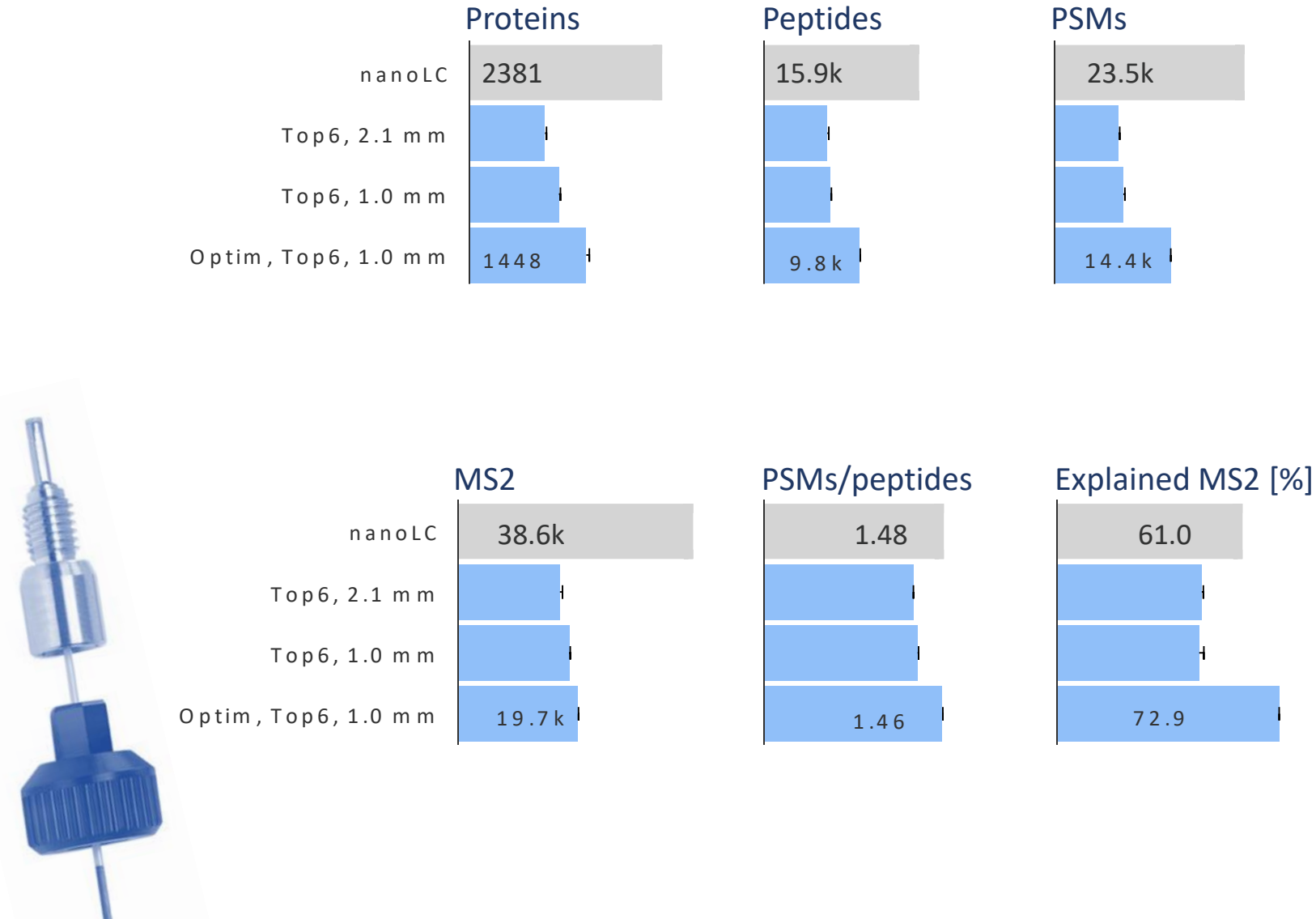


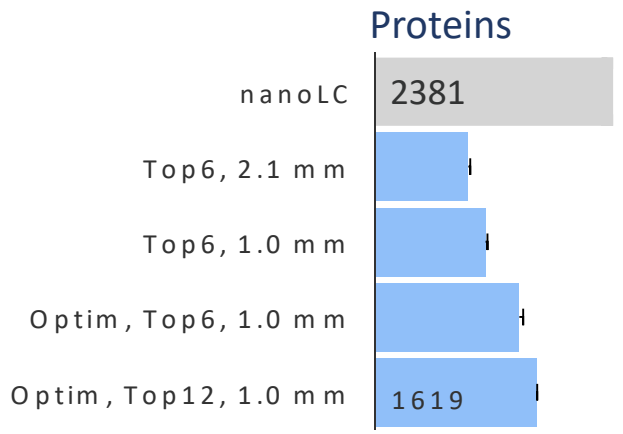
# Microbore LC

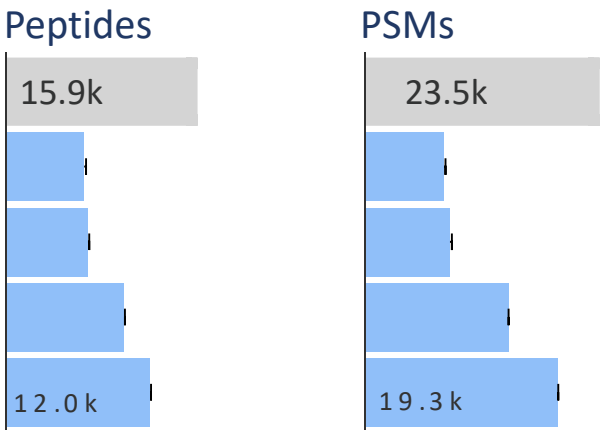
## 1.0 x 100 mm; 68 µl/min



# Microbore LC

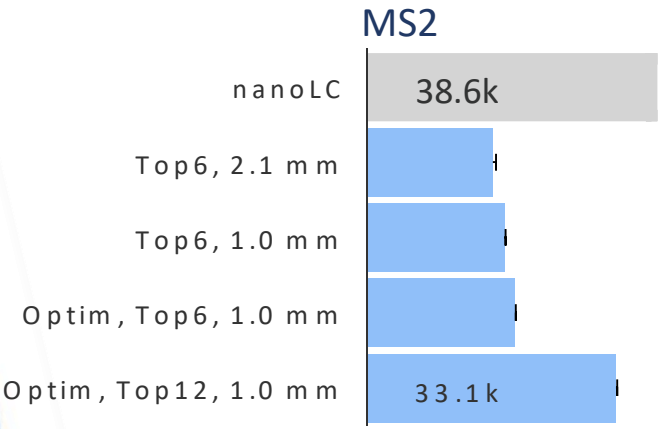
## 1.0 x 100 mm; 68 µl/min

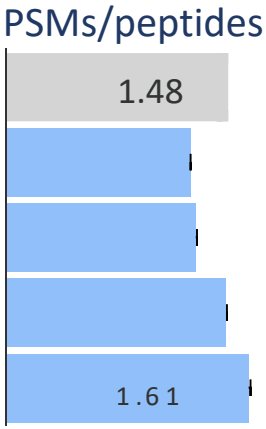


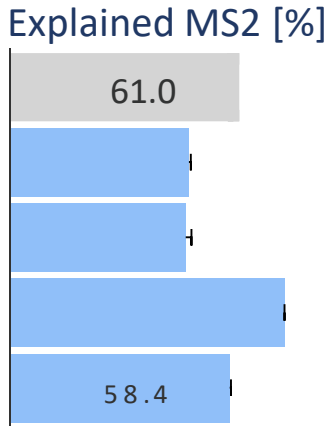







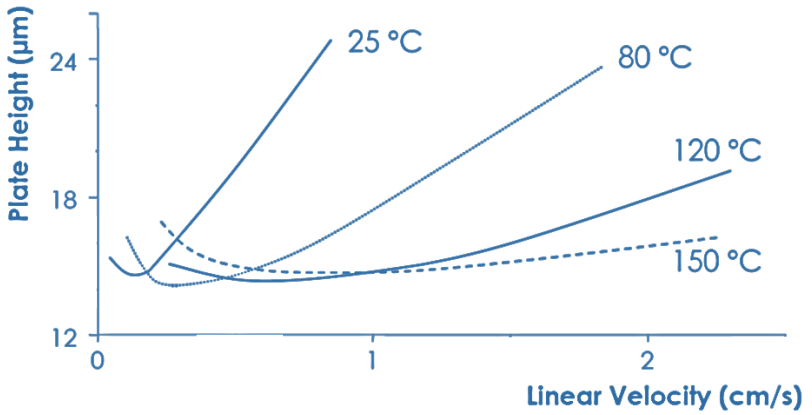


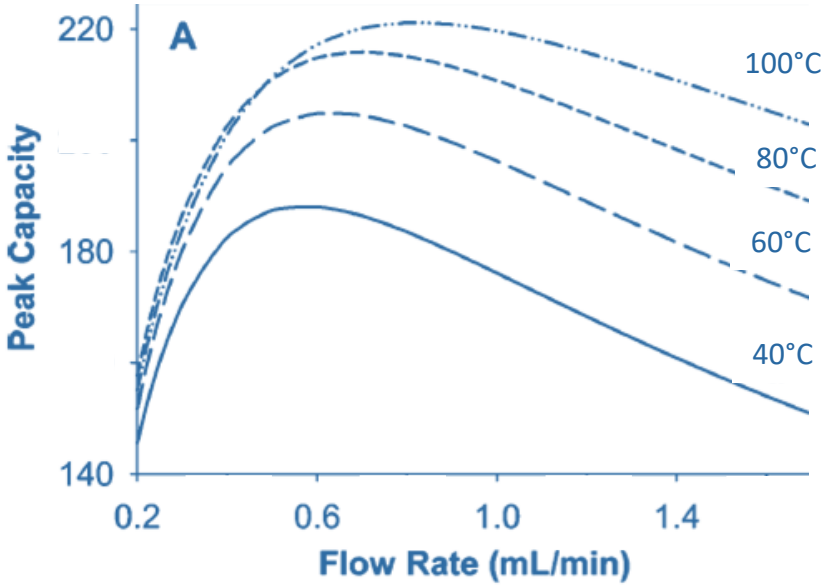



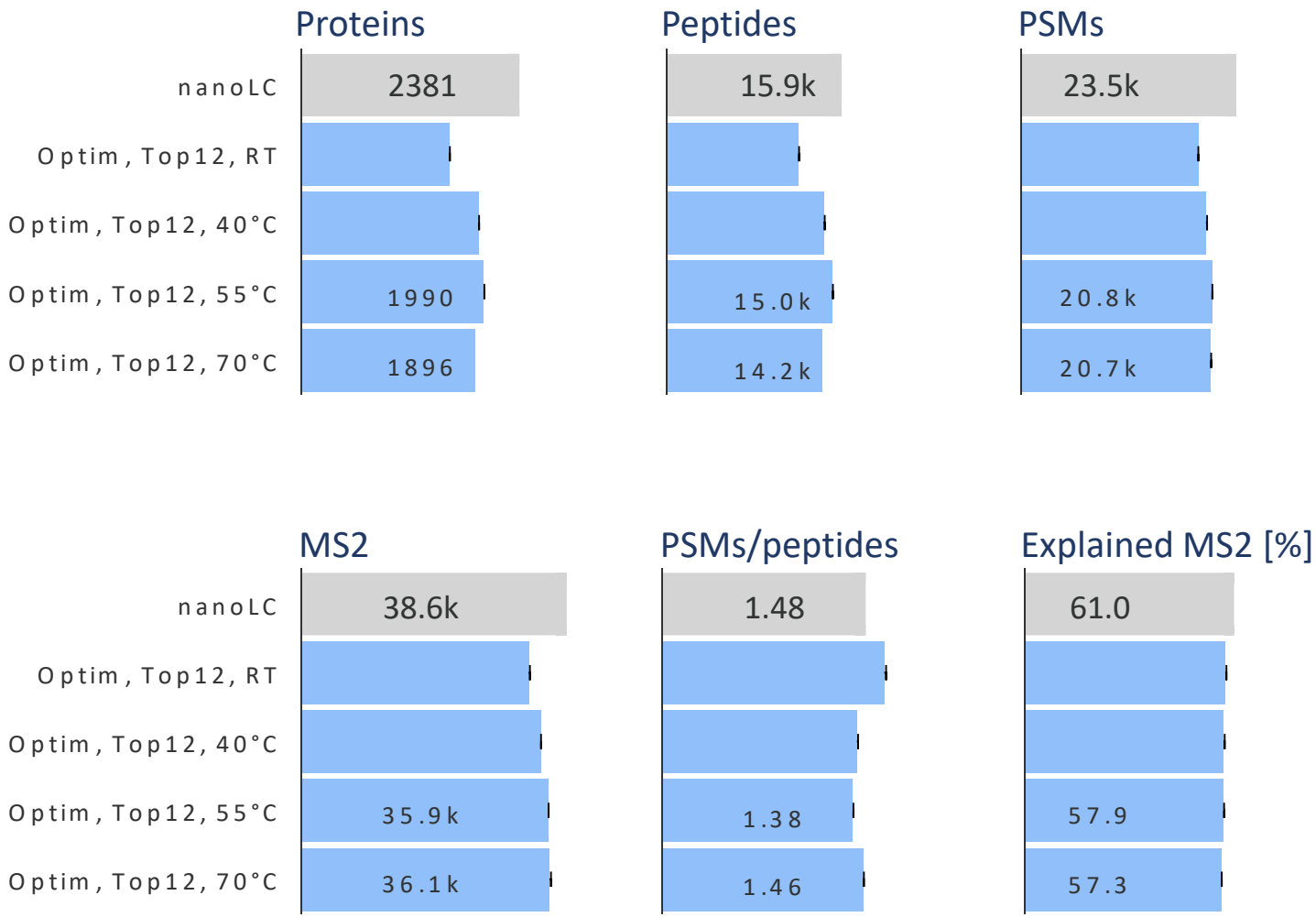
# Temperature

- ↘ η
  - ↘ pressure
  - ↗ $D_m$
- ↗ $D_m$
  - ↗ longitudinal diffusion
  - ↘ resistance to mass transfer
- ↗ second. interactions kinetics
- ↗ Pro isomerization kinetics
- ↘ carry-over



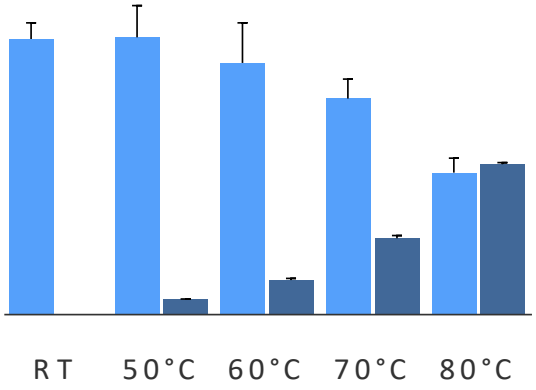



# Temperature

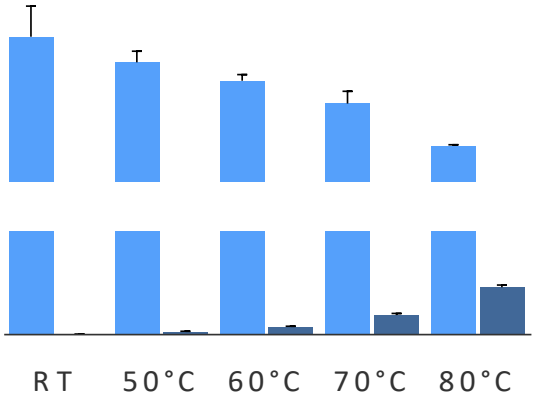


# Peptide degradation test

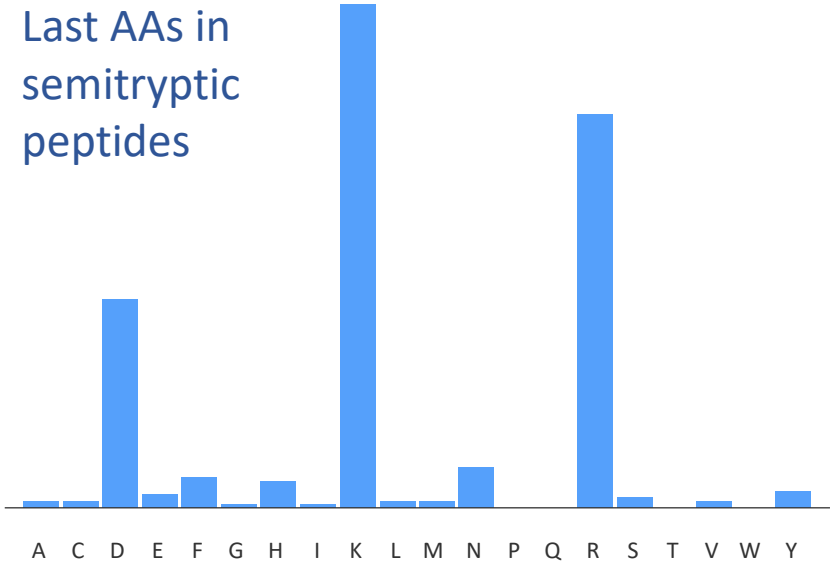
GIFPVLCKDPVQEAWAEDVDL
GIFPVLCKD




QEYDESGPSIVHR
ESGPSIVHR

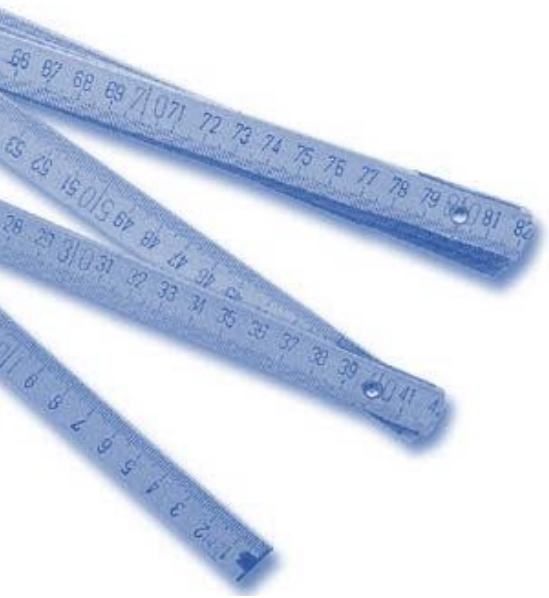


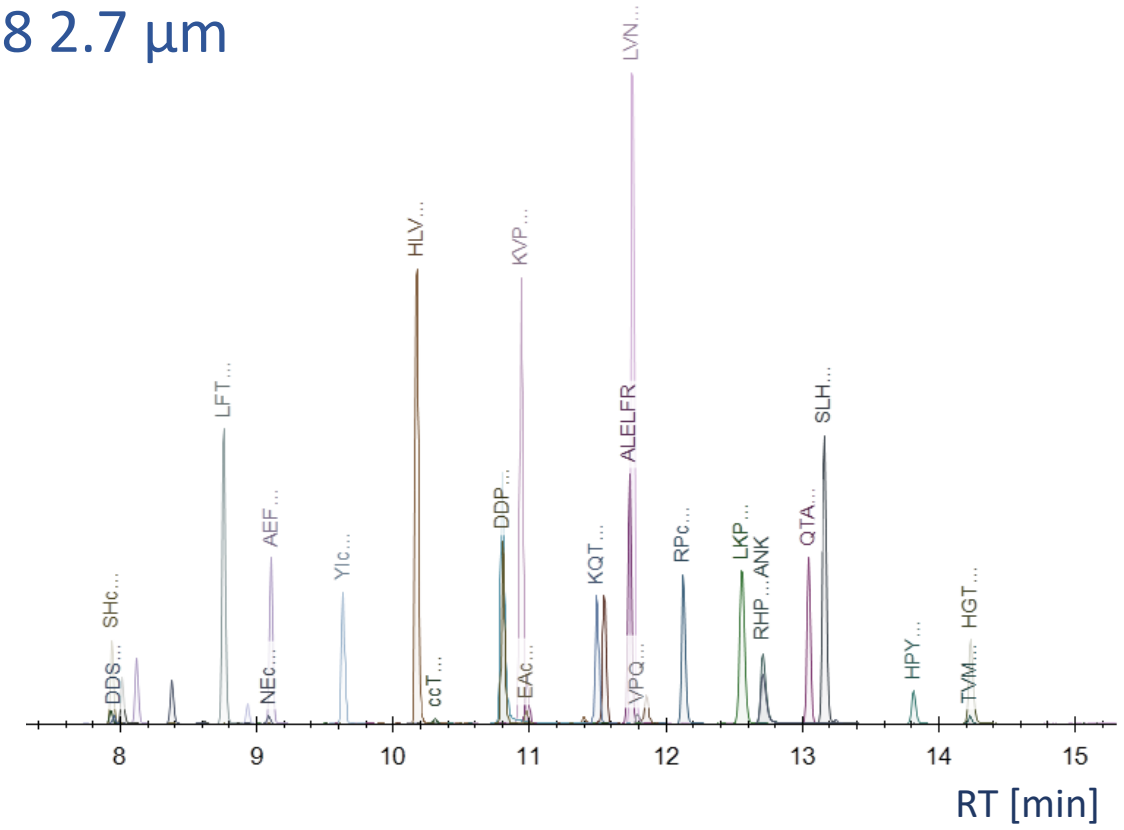

Last AAs in semitryptic peptides



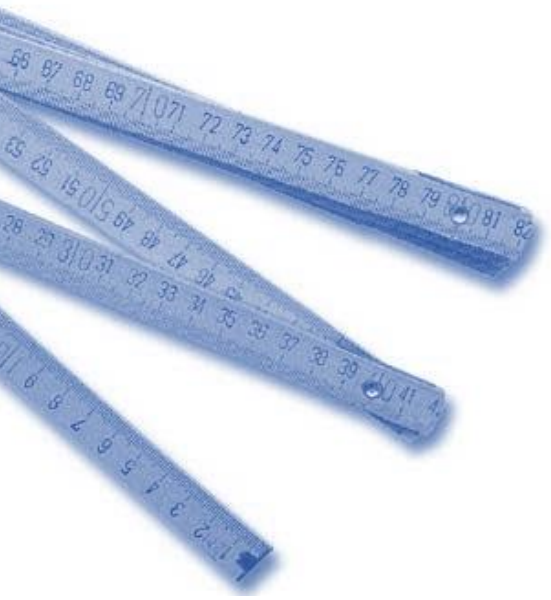
| Column length [mm] | Pc |
|---|---|
| 100 | 234 |
| 250 | 283 |

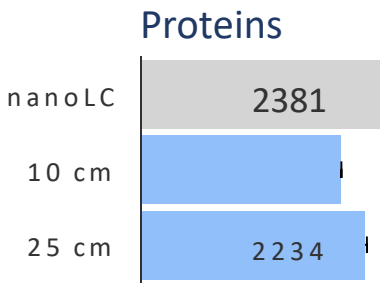
Halo Peptide 160Å C18 2.7 µm

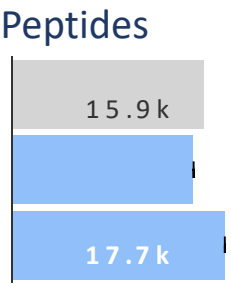


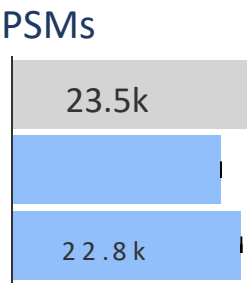


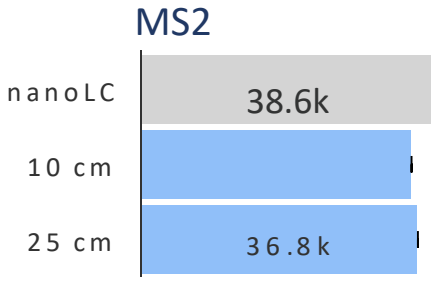
# Column length [mm]

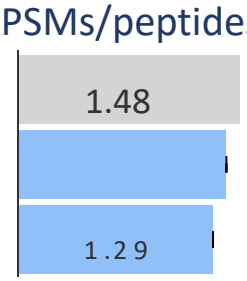


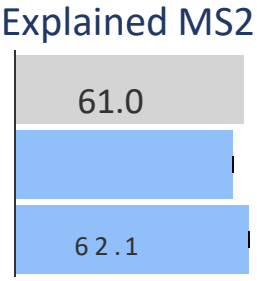


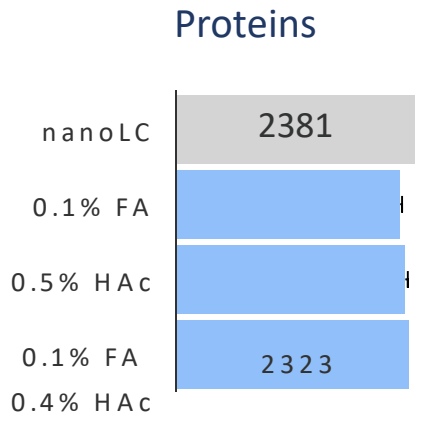



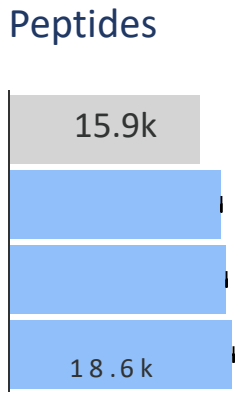



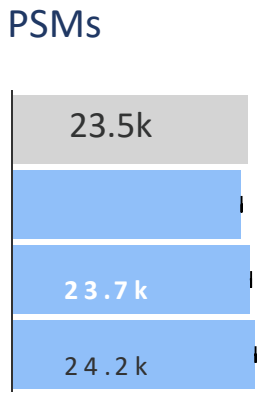



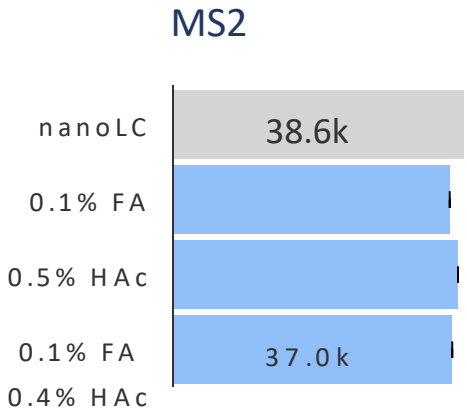
# Acidifiers

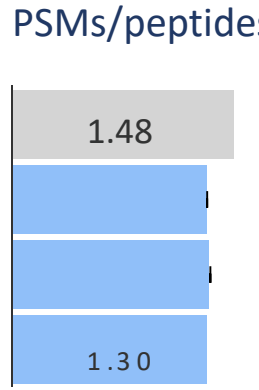

-PEPTIDES.COM
RESEARCH ONLY
Acetic Acid
10ML

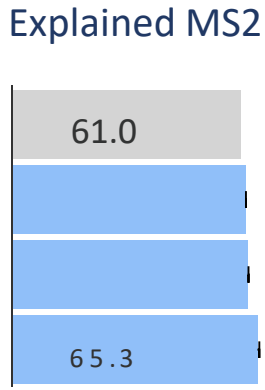

Proteins
nanoLC
0.1% FA
0.5% HAc
0.1% FA
0.4% HAc
2381
2323


Peptides
15.9k
18.6k


PSMs
23.5k
23.7k
24.2k

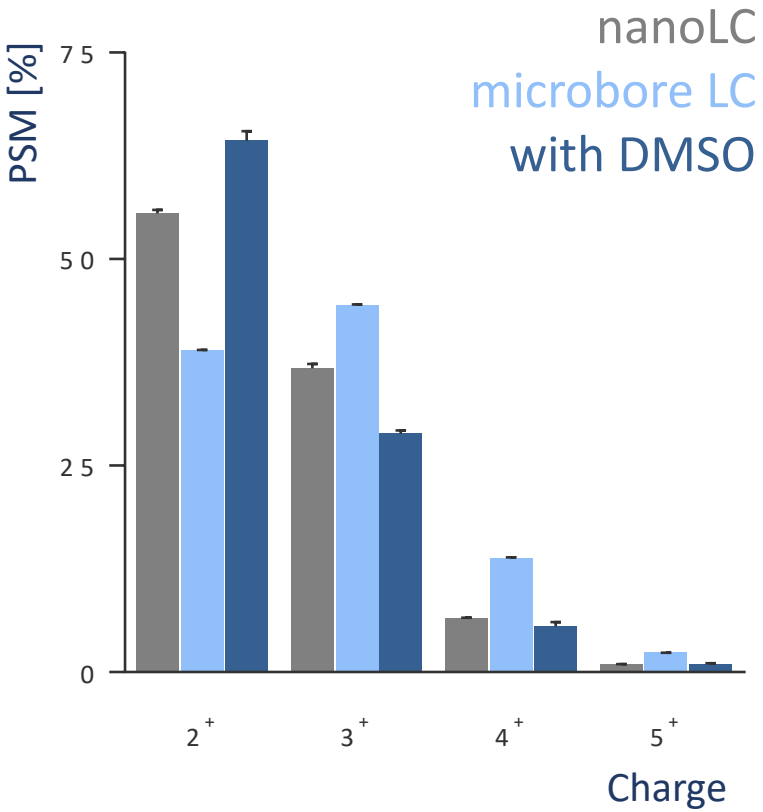

MS2
nanoLC
0.1% FA
0.5% HAc
0.1% FA
0.4% HAc
38.6k
37.0k

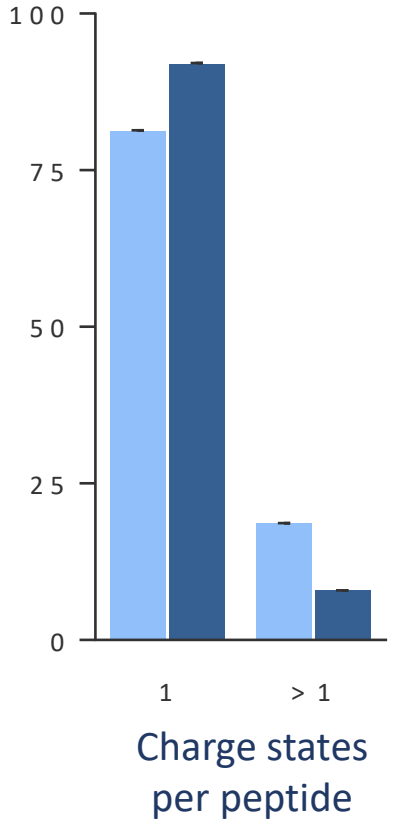

PSMs/peptides
1.48
1.30

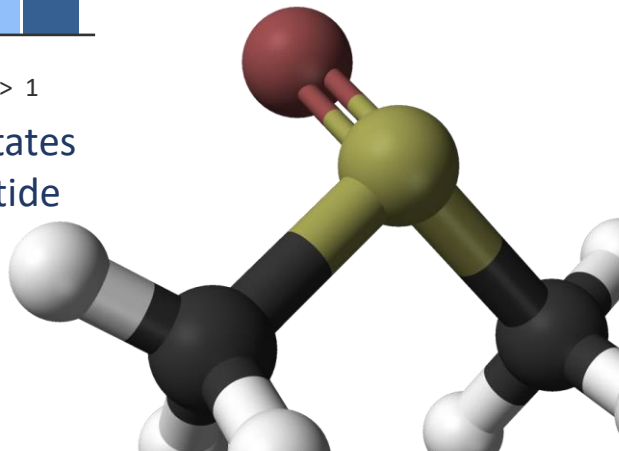

Explained MS2 [%]
61.0
65.3

# Additives

## for reduction of charge states

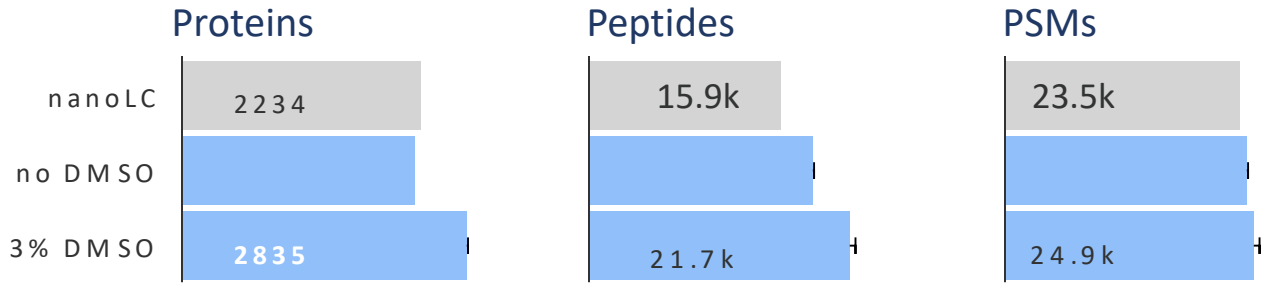


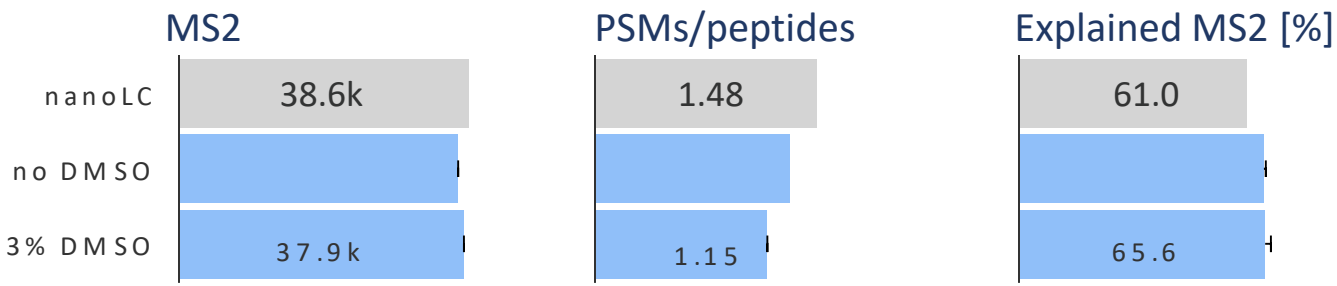



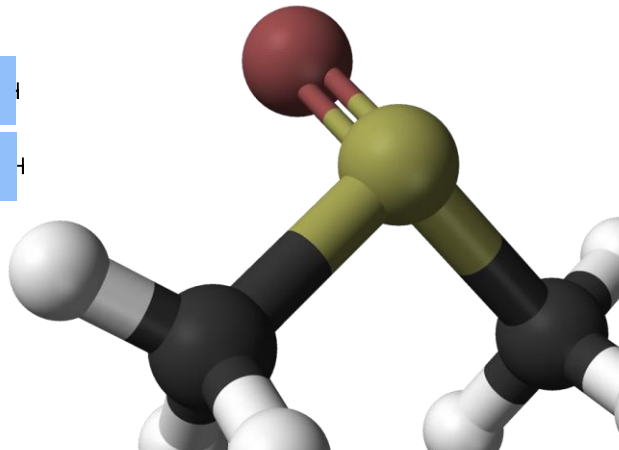



# Additives

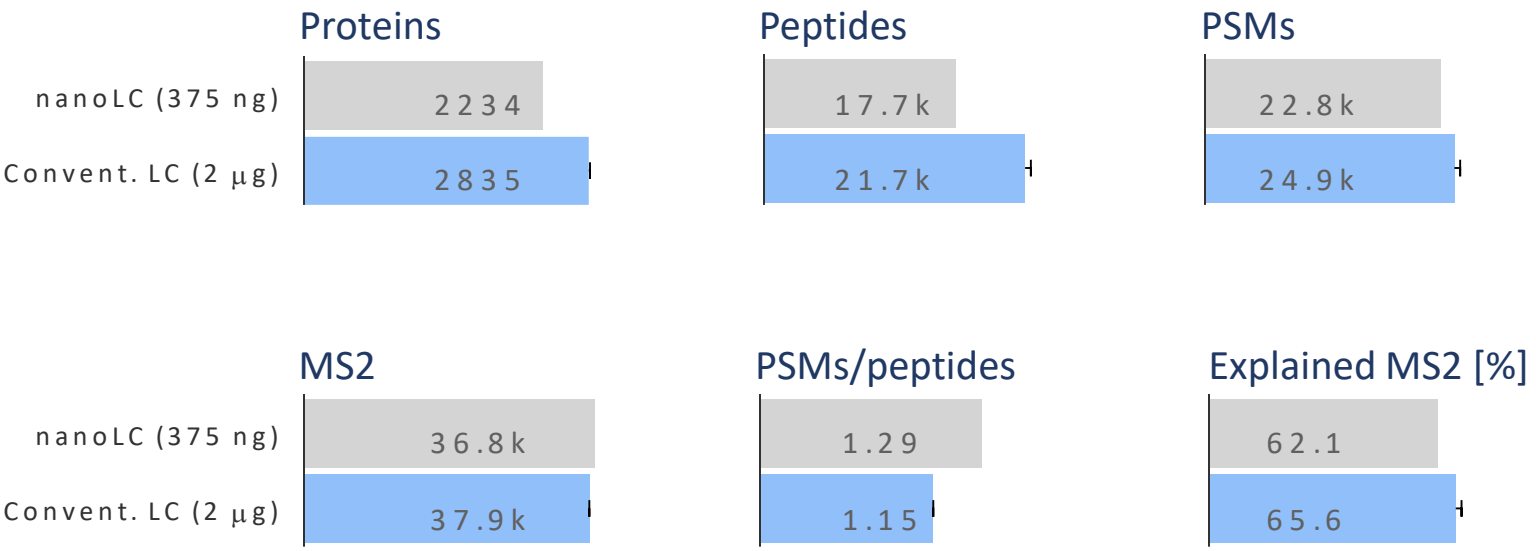
## for reduction of charge states



# Do we still need nanospray for exploratory proteomics?




0.075 x 250 mm
250 nL/min
22 °C
0.1% FA

1.0 x 250 mm
68 µL/min
55 °C
3% DMSO, 0.1/0.4% FA/Hac
$\sigma_{ec}^2$

STARSS

EUROPEAN UNION
European Structural and Investment Funds
Operational Programme Research, Development and Education

MINISTRY OF EDUCATION, YOUTH AND SPORTS

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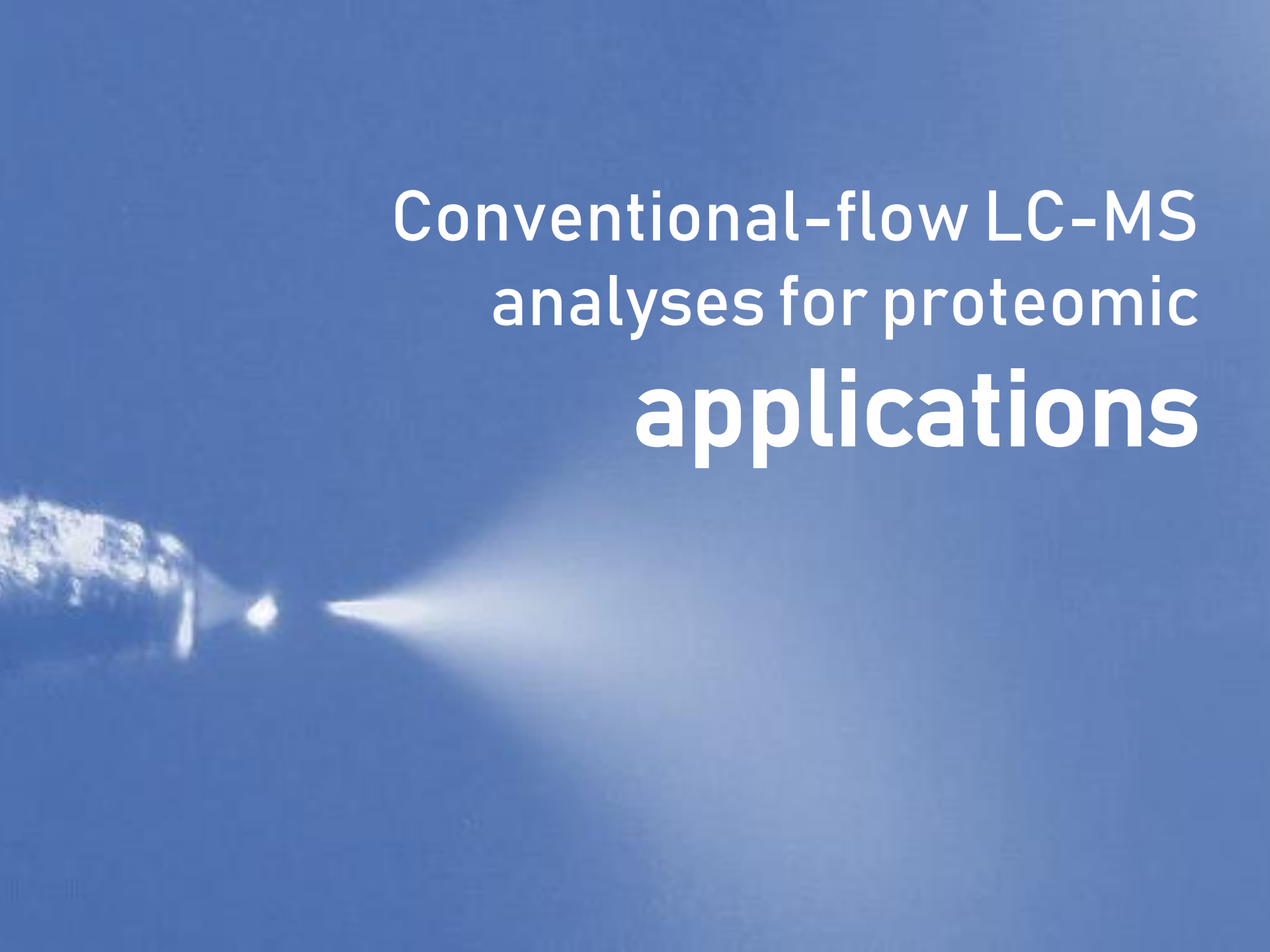
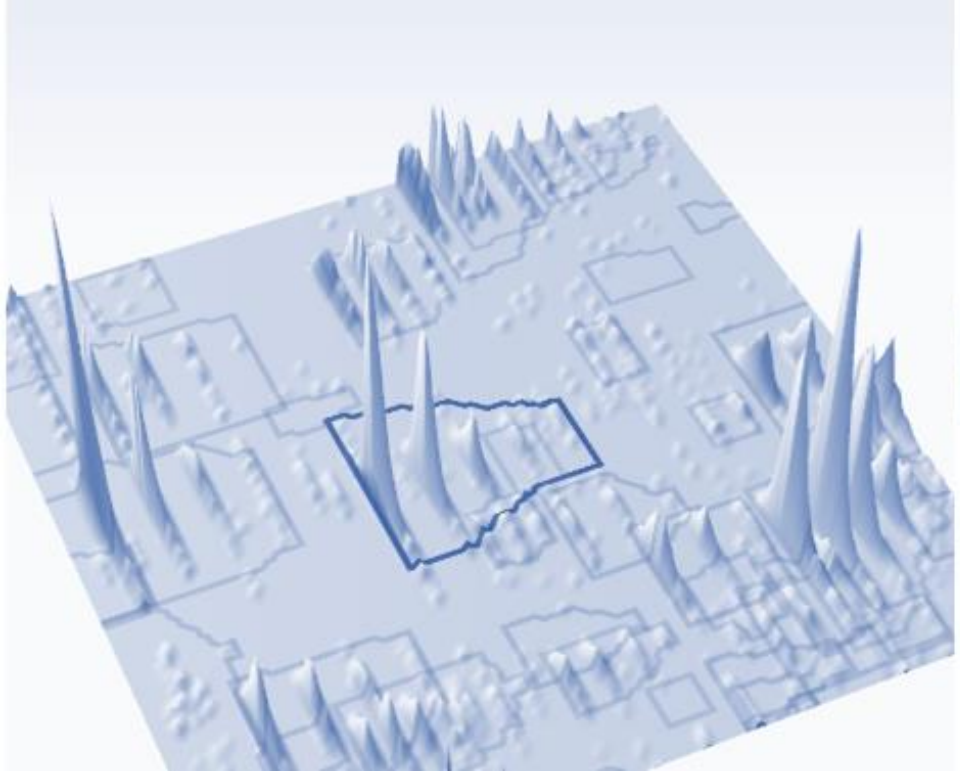
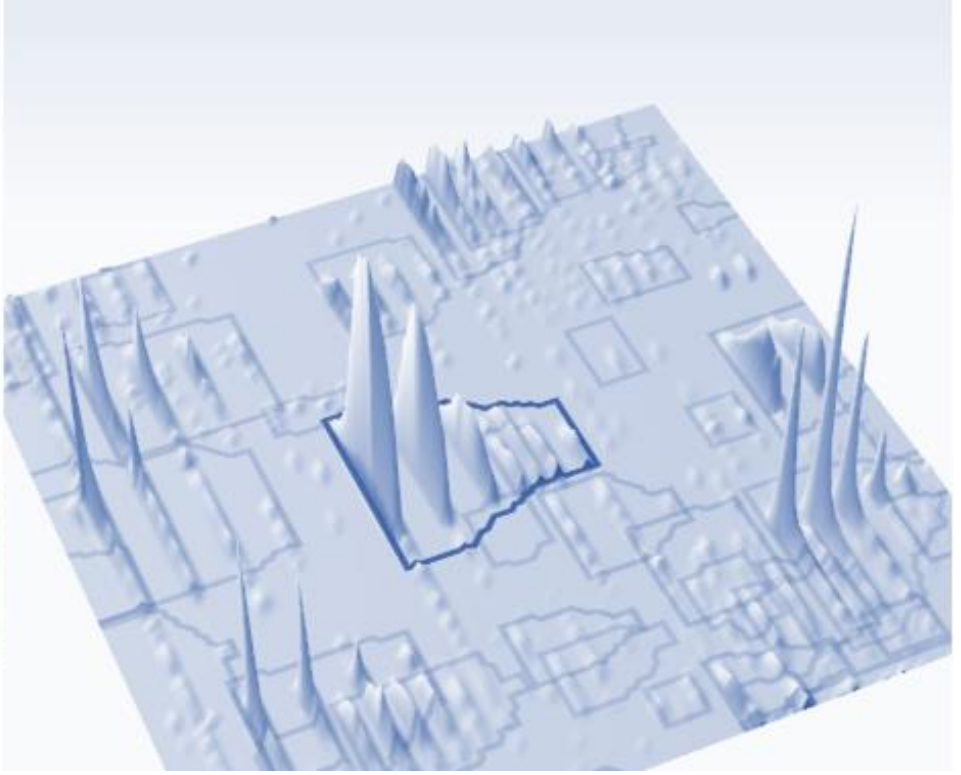
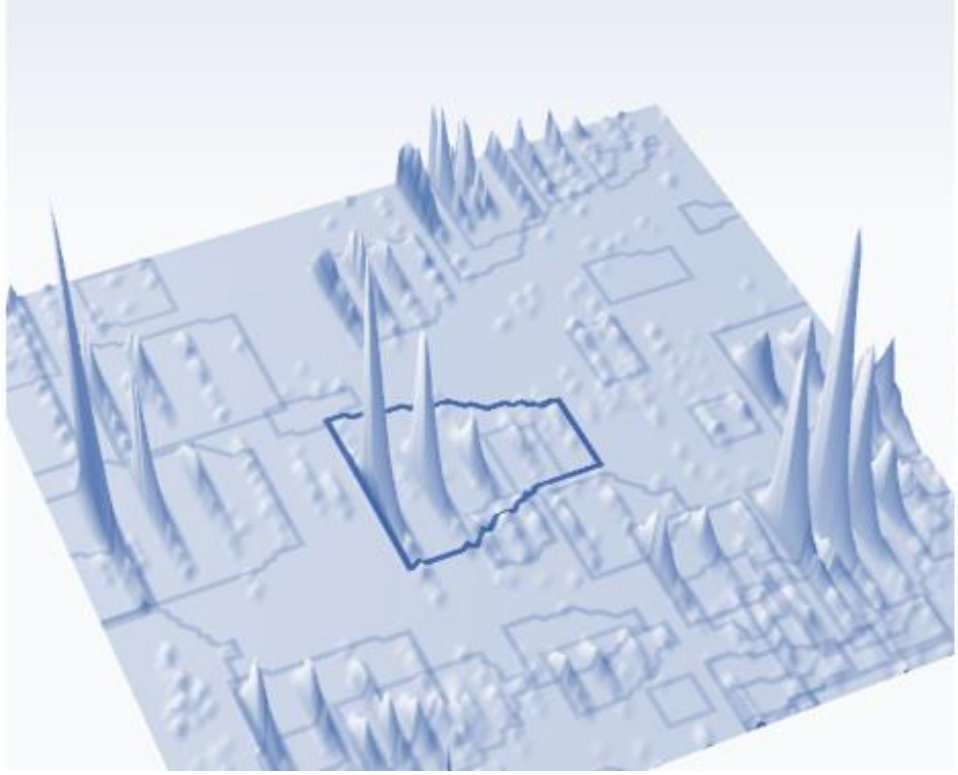
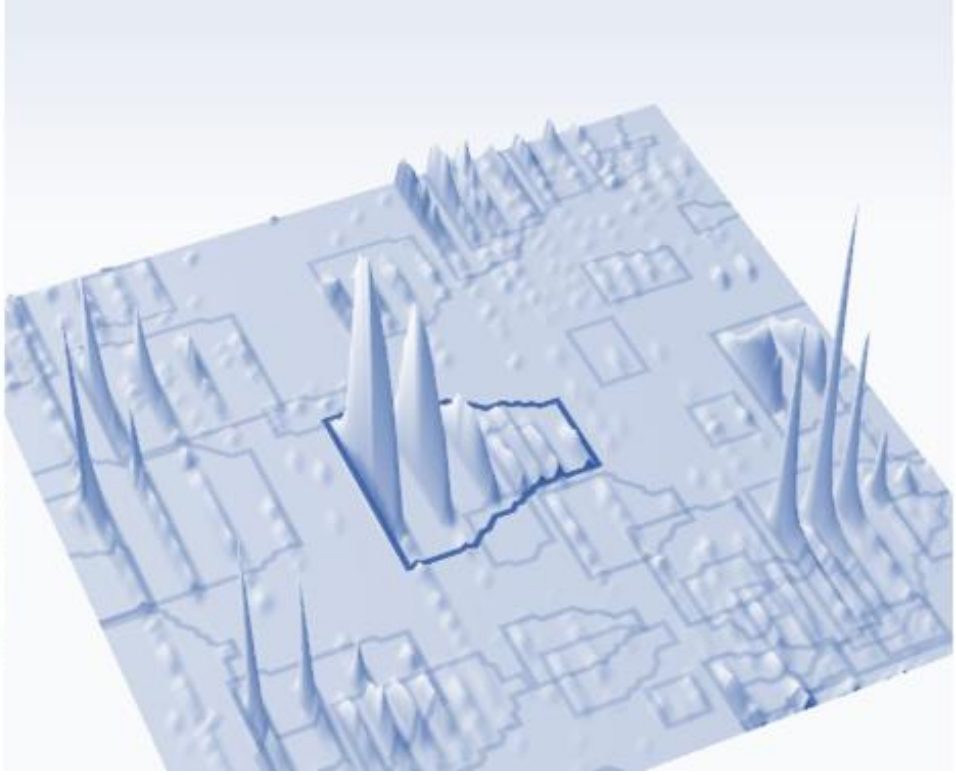

Conventional-flow LC-MS
analyses for proteomic
applications





# Label-free MS1 quantification



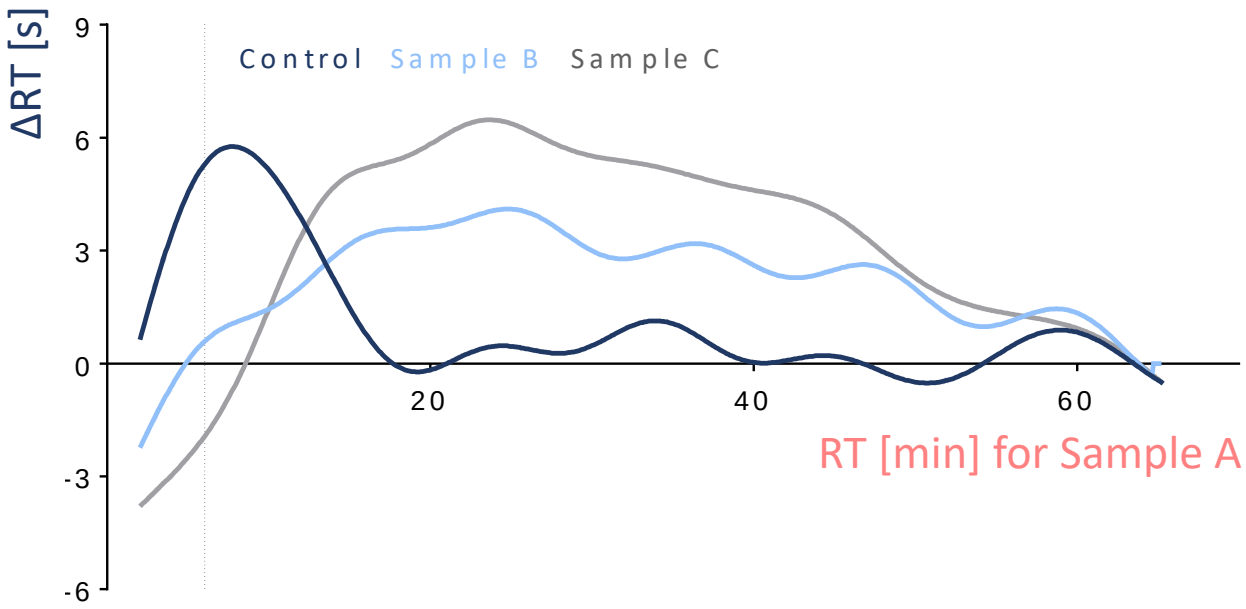


**Control**
1000 ng bacterial
in 2000 ng background

Sample A
1000 ng
in 2000 ng

Sample B
316 ng
in 2000 ng

Sample C
100 ng
in 2000 ng




**Control**
1000 ng bacterial
in 2000 ng background

Sample A
1000 ng
in 2000 ng

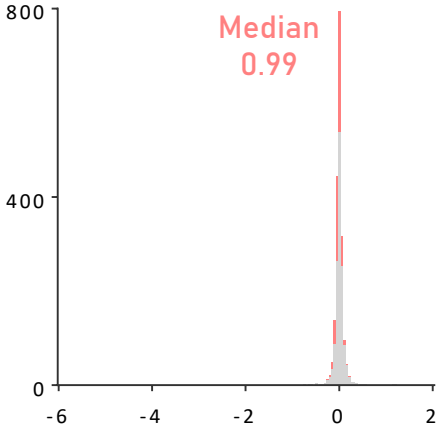
Sample B
316 ng
in 2000 ng

Sample C
100 ng
in 2000 ng

## Sample A

1000 ng

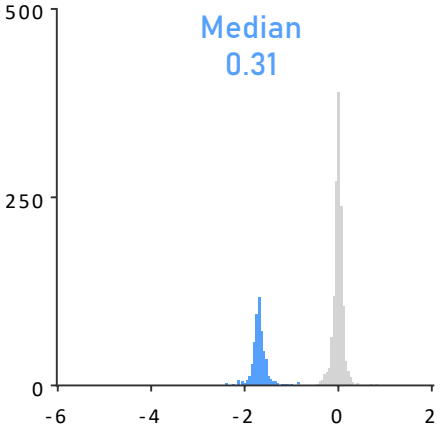
in 2000 ng




## Sample B

316 ng

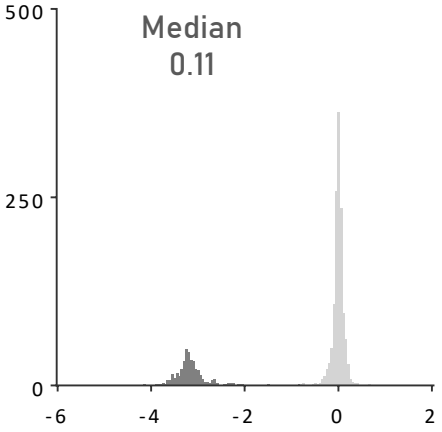
in 2000 ng




## Sample C

100 ng

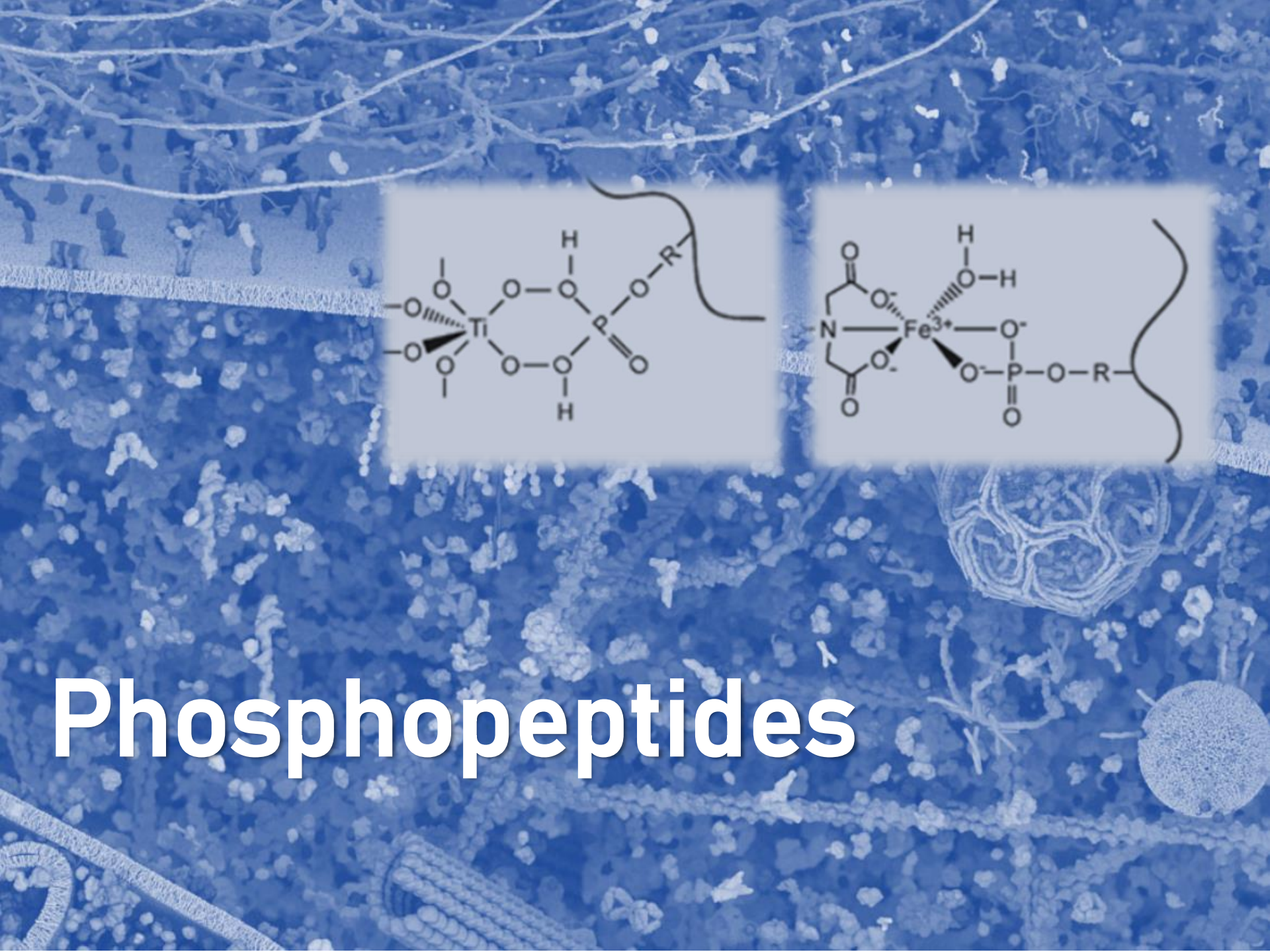
in 2000 ng



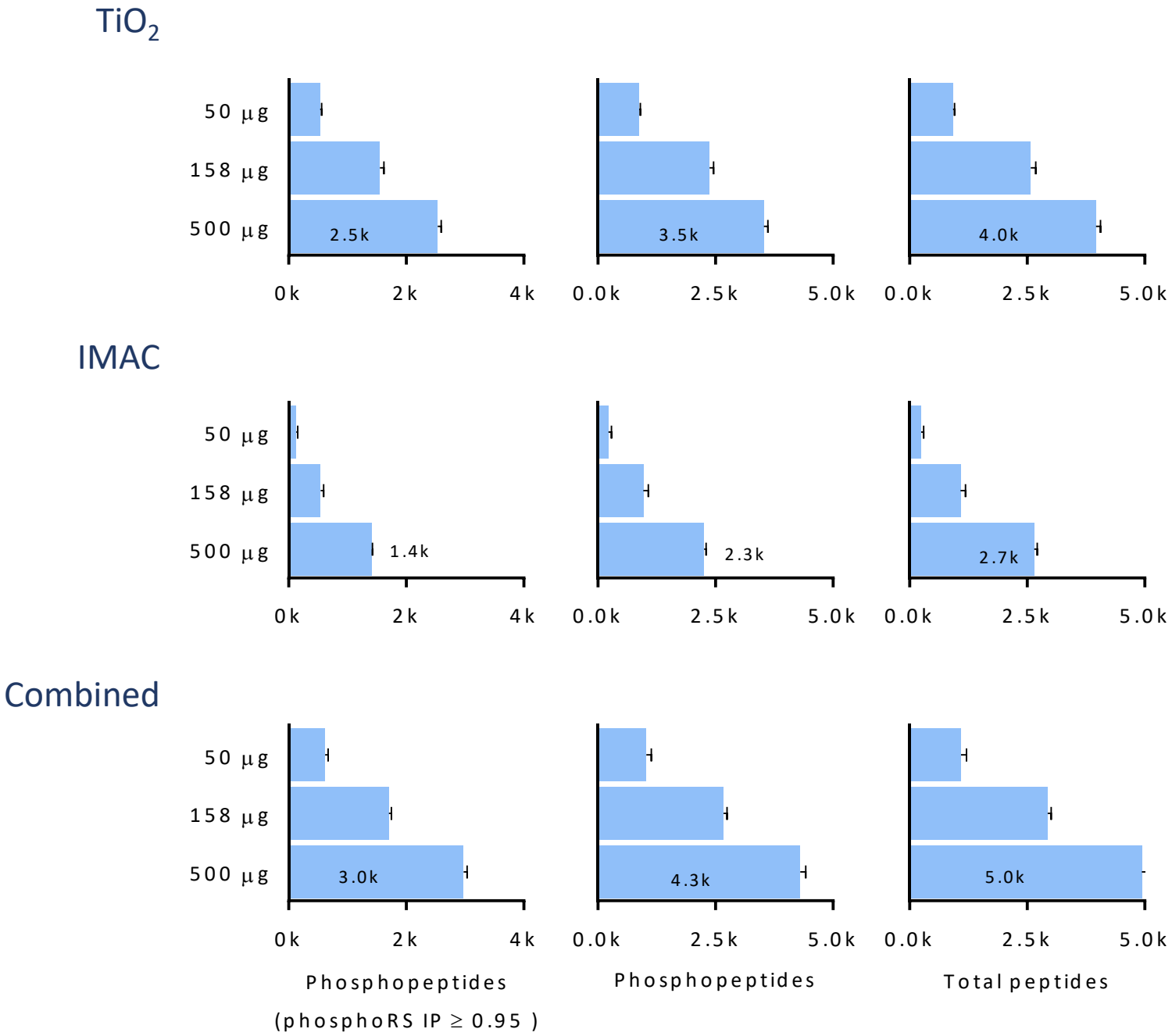

## Control

1000 ng bacterial

in 2000 ng background




# Phosphopeptides


TiO2
50 µg
158 µg
500 µg
2.5k
3.5k
4.0k
0k
2k
4k
0.0k
2.5k
5.0k
IMAC
1.4k
2.3k
2.7k
Combined
3.0k
4.3k
5.0k
Phosphopeptides
(phosphoRS IP ≥ 0.95 )
Phosphopeptides
Total peptides

# Conventional-Flow Liquid Chromatography–Mass Spectrometry for Exploratory Bottom-Up Proteomic Analyses

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