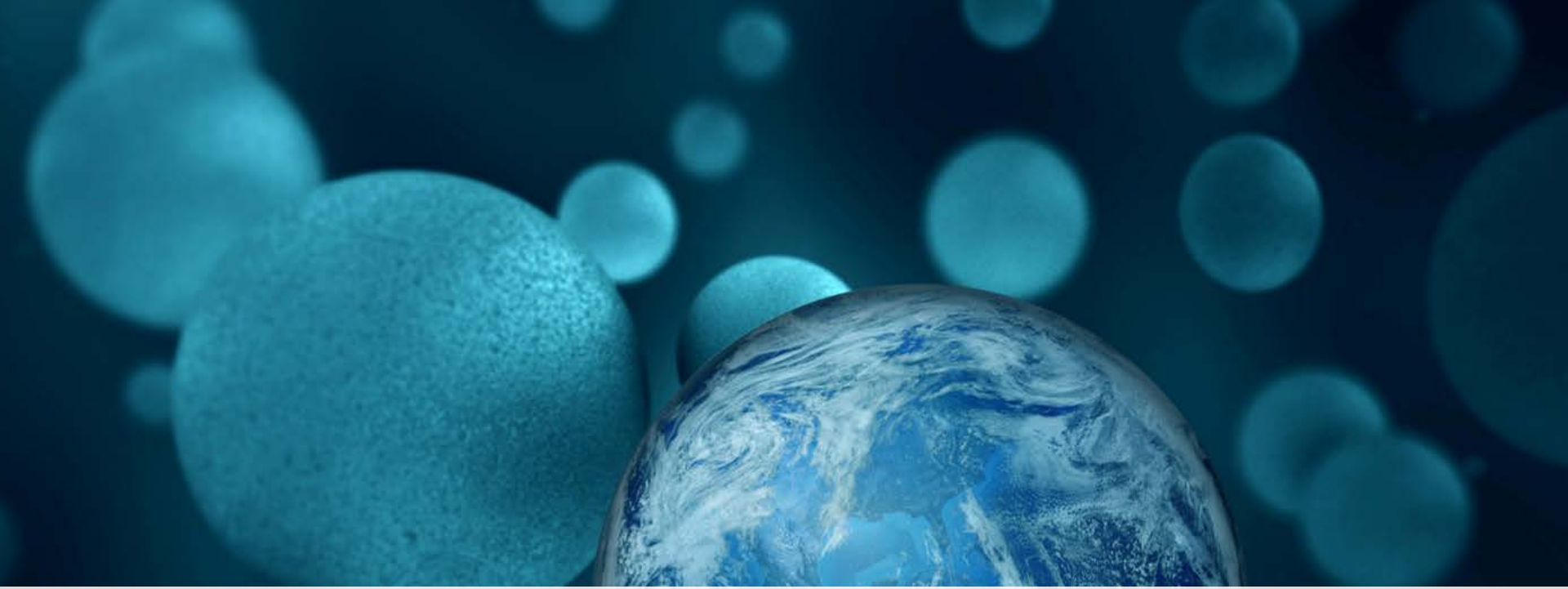


ThermoFisher SCIENTIFIC

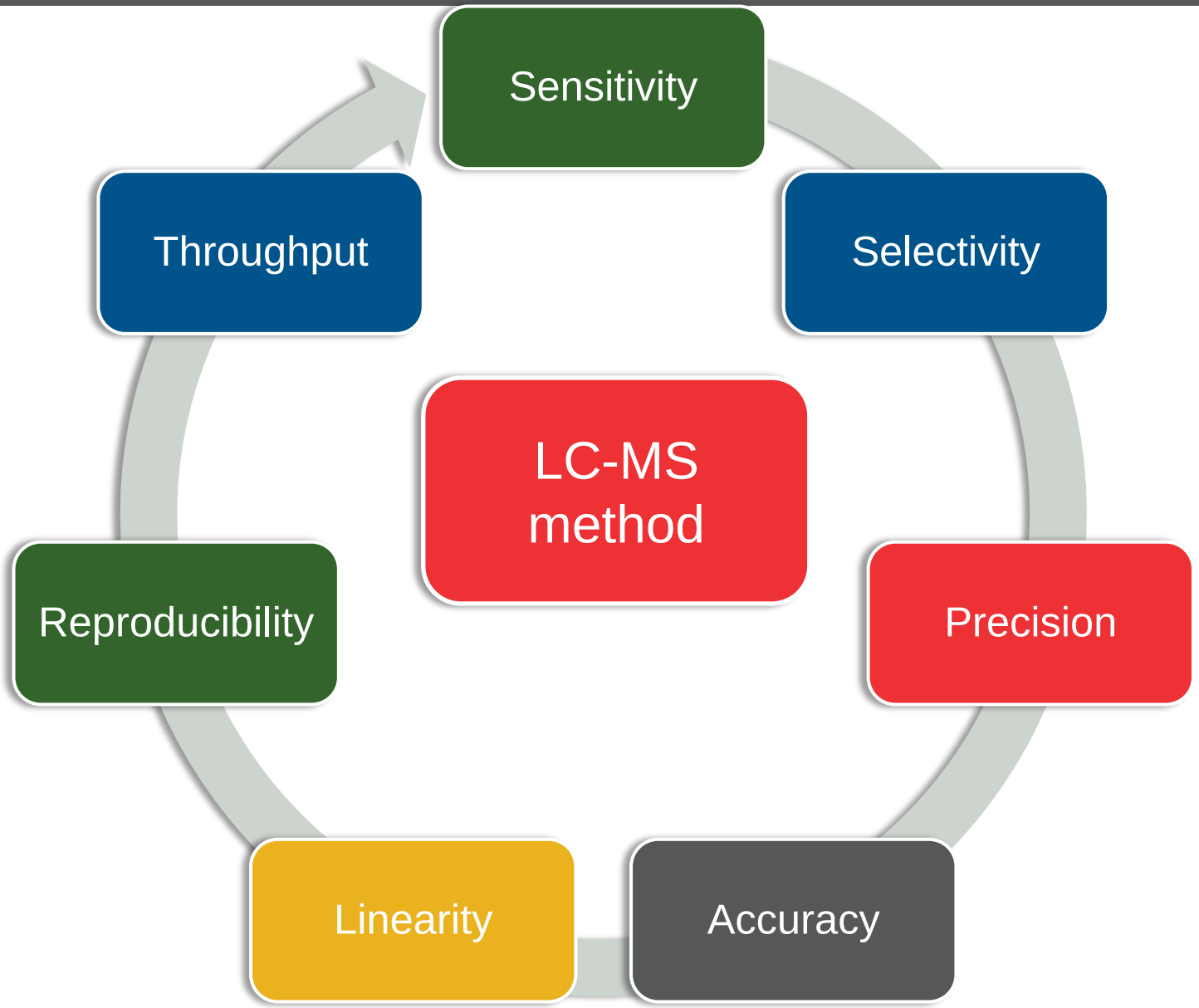
From analytical to nano-flow LC-MS: high robustness and sensitivity to answer complex biological questions

2017-03-26 Alexander Boychenko, Remco Swart

Content

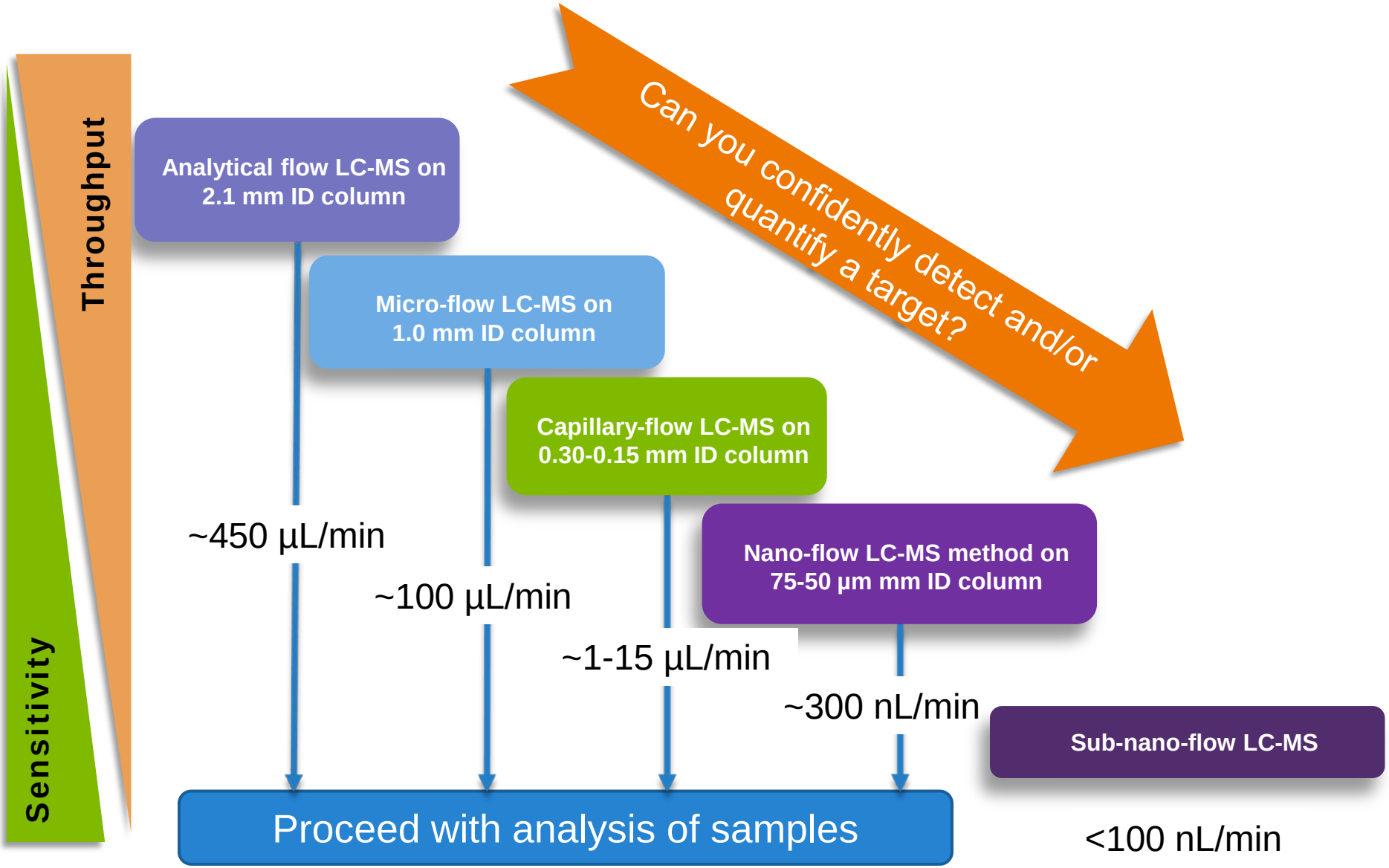
- LC-MS sensitivity gains and tailored method selection
- Capillary and micro-flow LC-MS: what to expect?
- Nano-flow LC-MS: the gold standard in proteomics
- Why chromatography is important for LC-MS proteomics
- Nano-flow and sub-nano flow rates

LC-MS method development



ThermoFisher SCIENTIFIC

When do you need to downscale your chromatography?



ThermoFisher SCIENTIFIC

LC-MS with ESI: flow rate and sensitivity gain

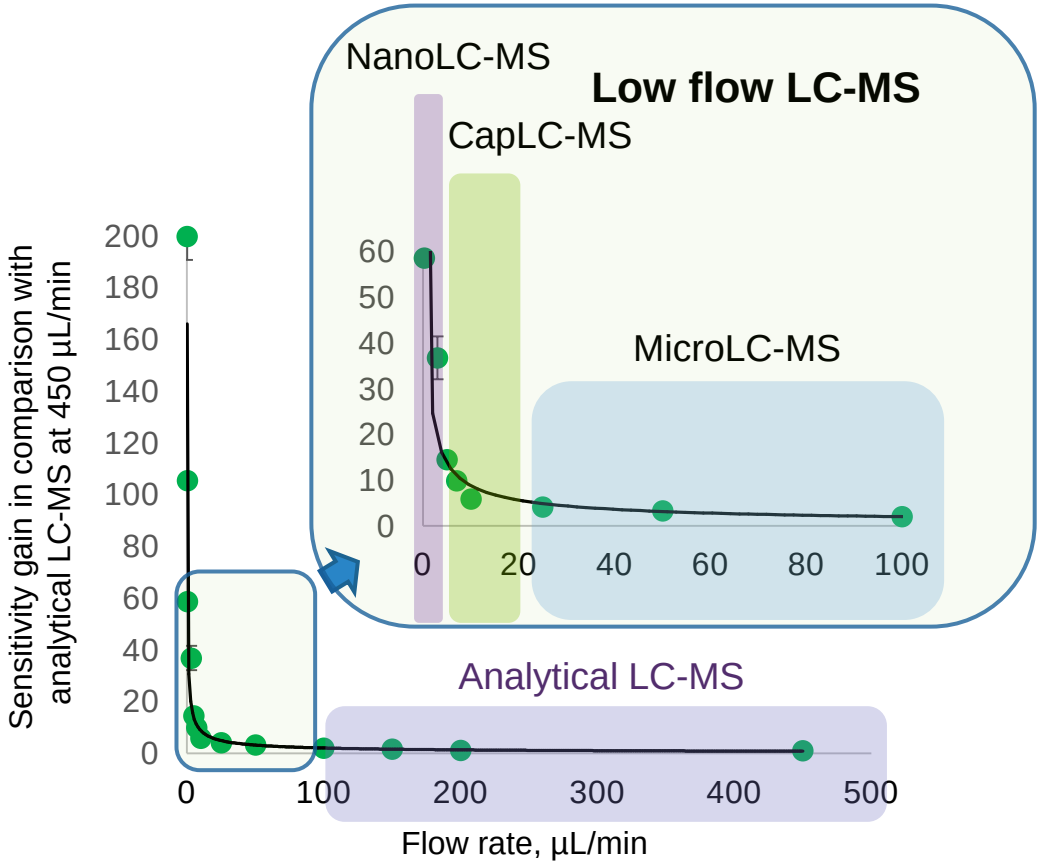
ESI-MS exhibits a mixed behavior of both concentration and mass-sensitive detectors

Sensitivity gains (experimental, relative to 2.1 mm ID)

microLC-MS:	2-4
capLC-MS:	4-50
nanoLC-MS:	> 50
sub-nanoLC-MS:	> 100

Reasons

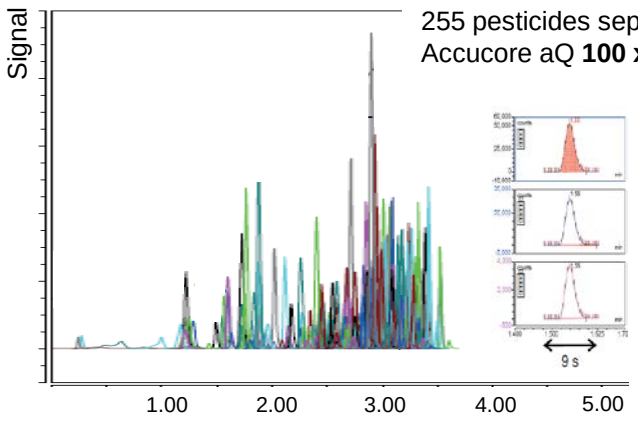
- Increase of analyte concentration with decrease of column ID
- Improved ionization efficiency



The sensitivity gains were measured as a relative peak area averaged for Cytochrome C tryptic peptides

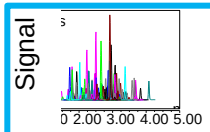
ThermoFisher SCIENTIFIC

From analytical to micro-flow LC-MS



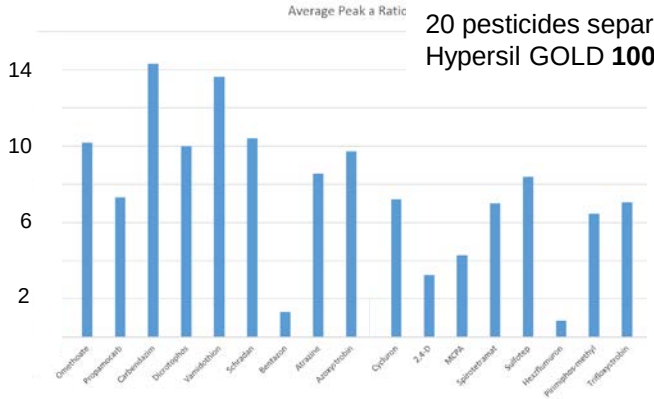
255 pesticides separated on
Accucore aQ **100 x 2.1 mm**, 2.6 µm

- ✓ Ultrafast UHPLC-MS SRM method
- ✓ > 500 SRMs with positive/negative
- ✓ Retention time standard deviation < 0.3 sec



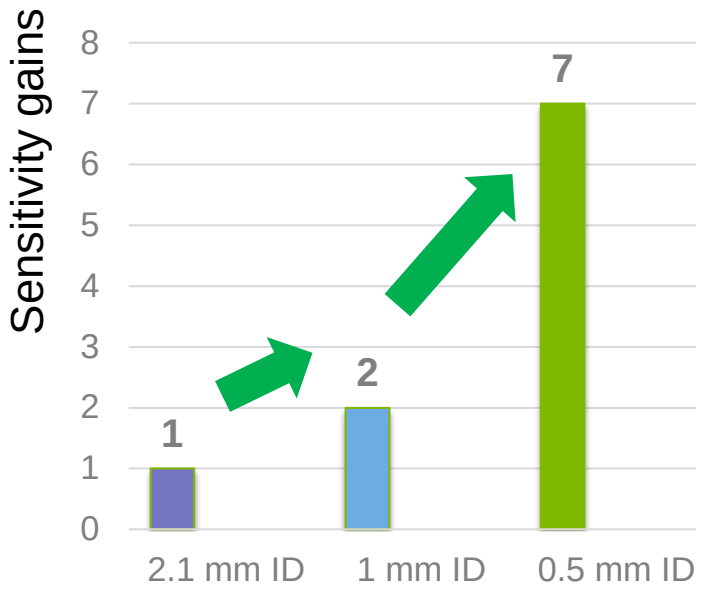
255 pesticides separated on
Hypersil GOLD **100 x 1.0 mm**, 1.9 µm

- ✓ Same throughput and robustness as for 2.1 mm ID
- ✓ 2 times sensitivity gain on average



20 pesticides separated on
Hypersil GOLD **100 x 0.5 mm**, 1.9 µm

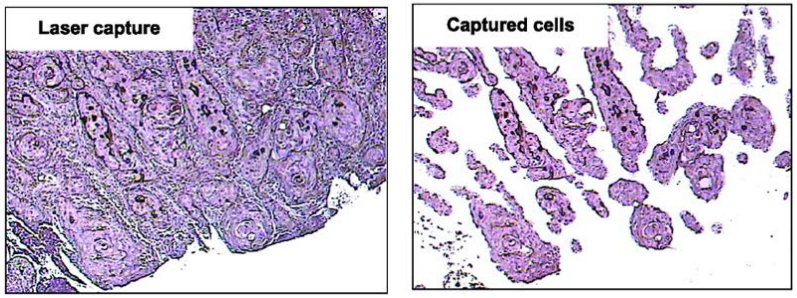
- ✓ **7 times** sensitivity gain on average
- ✓ Increased analysis time (15 min)



ThermoFisher SCIENTIFIC

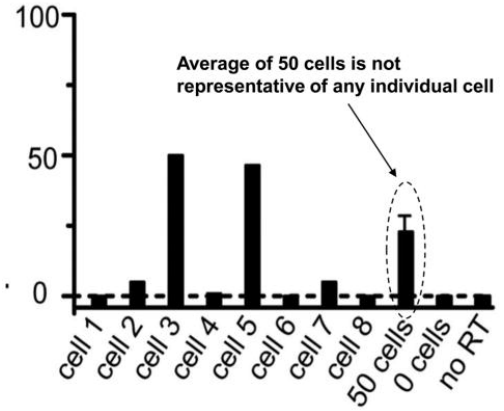
Why do we need nanoLC-MS?

- Extremely low amount of sample (e.g. laser micro-disected cells, small animal bio-fluids)
- Tumor biopsies
- Low analyte concentration in a complex matrix (biomarkers)
- High sample complexity and wide dynamic range
- Single cell -omics



LMD captured cells

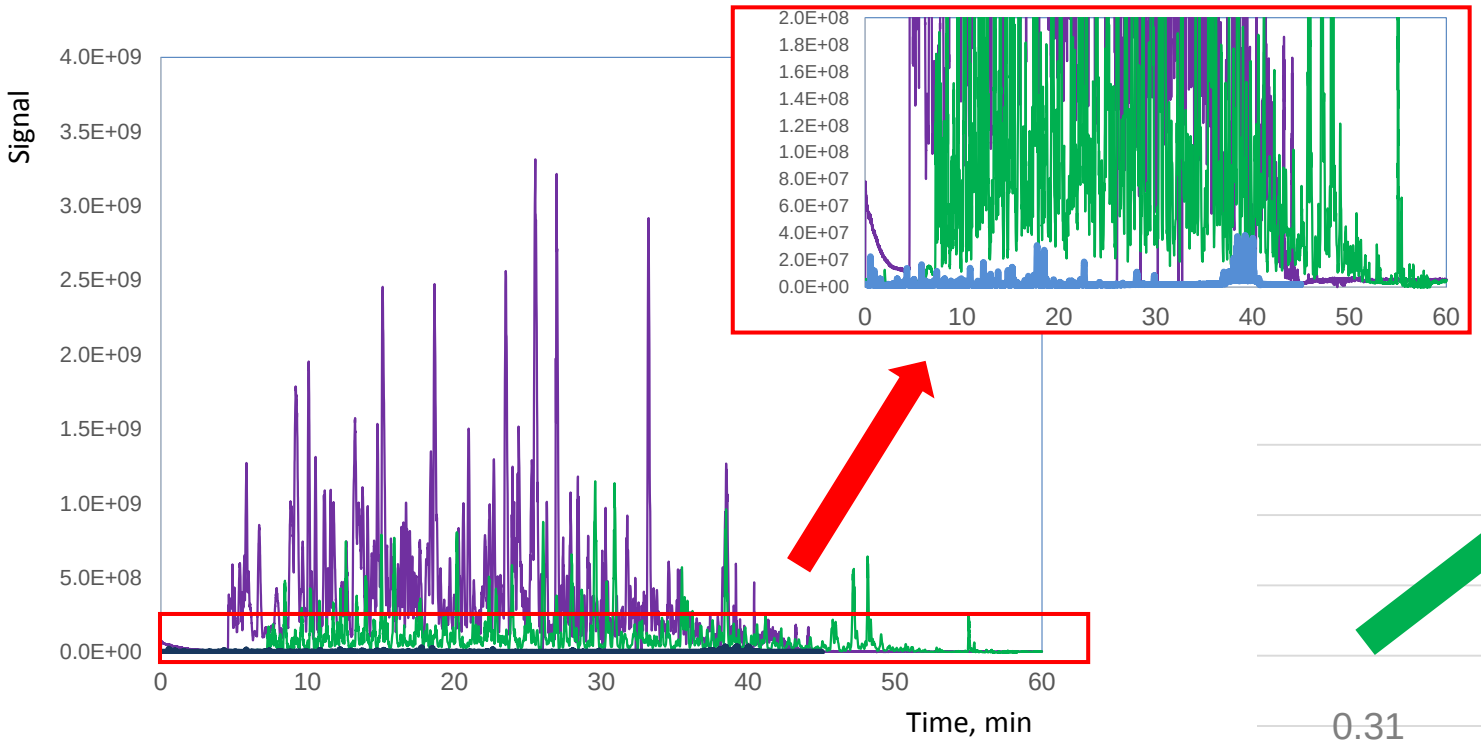
Patel et al. doi: 10.1158/1078-0432.CCR-07-1497



mRNA expression of GAPDH from individual Jurkat cells

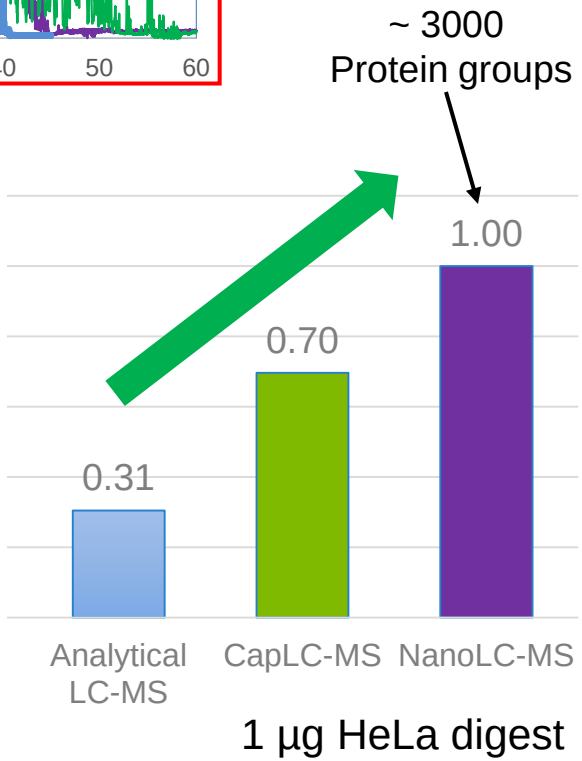
Toriello et al. doi: 10.1073/pnas.0806355106

NanoLC-MS is a gold standard in proteomics



Base peak chromatograms of HeLa cell lysate digest (1 µg)

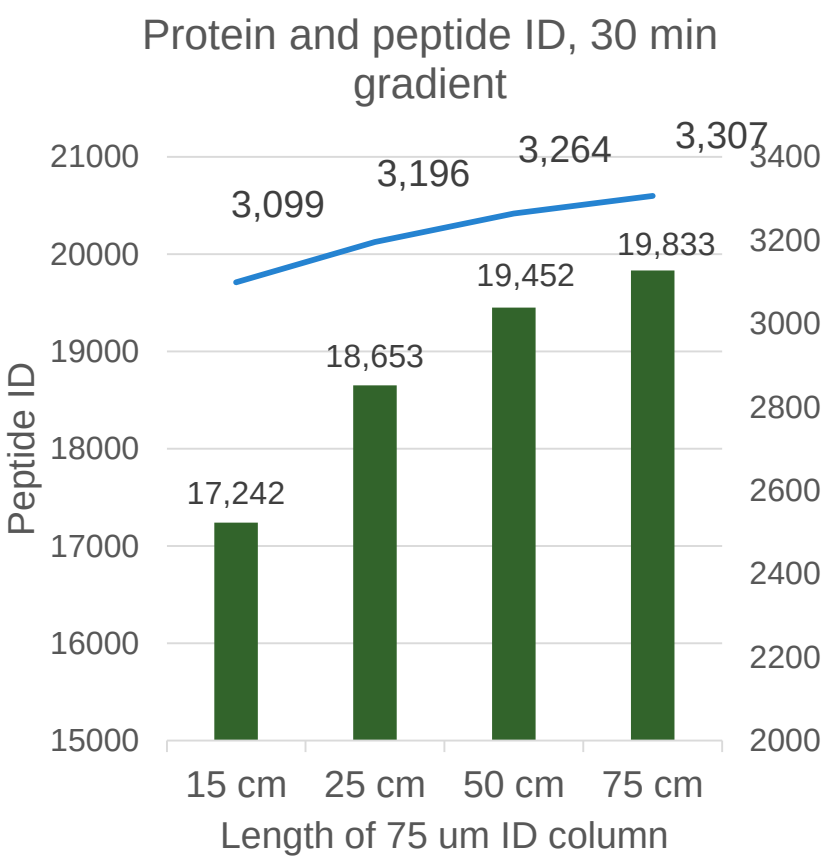
analytical flow LC-MS: blue
capLC-MS: green
nanoLC-MS: purple

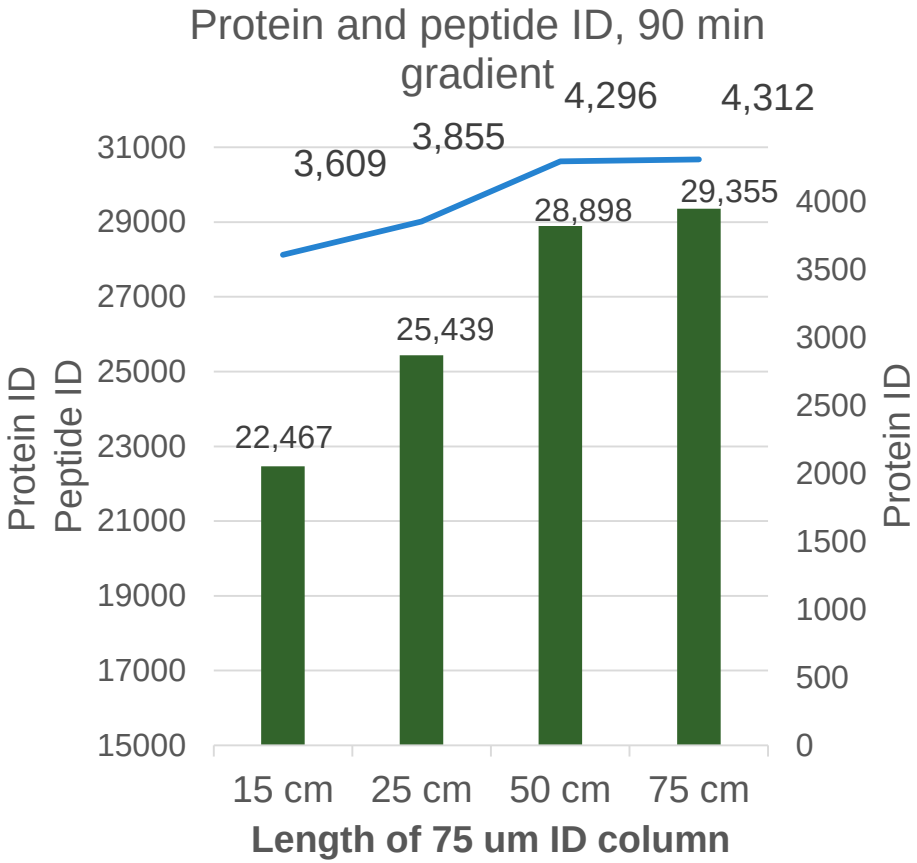


✓ High LC-MS sensitivity is essential for deep proteomics

ThermoFisher SCIENTIFIC

NanoLC-MS proteomics: the effect of column length



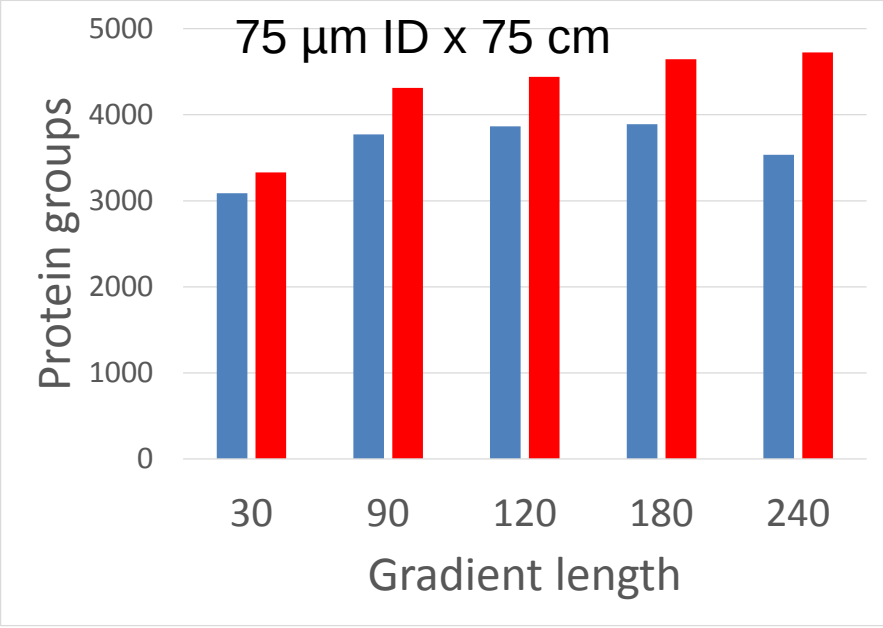


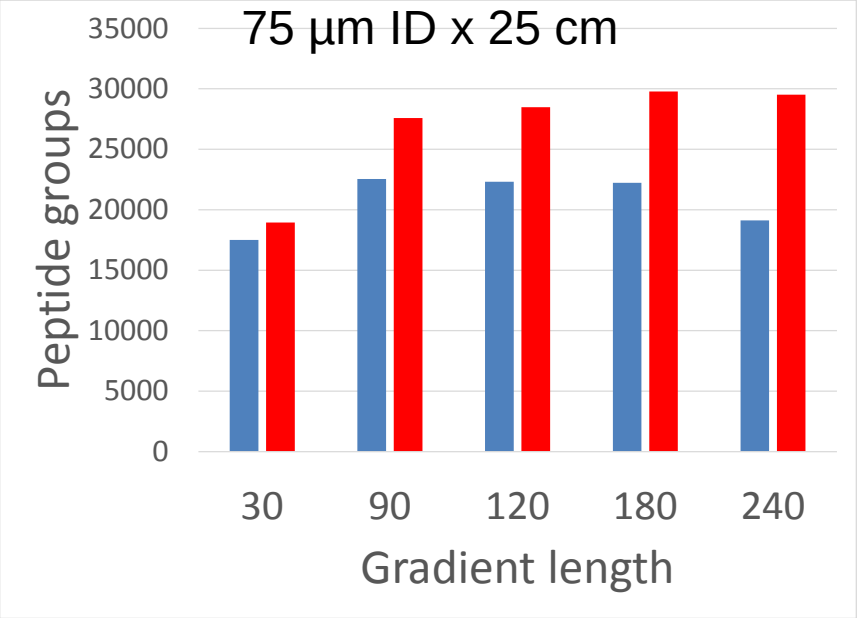
Protein ID: blue line
Peptide ID: green bars

✓ Deeper proteome coverage with longer columns

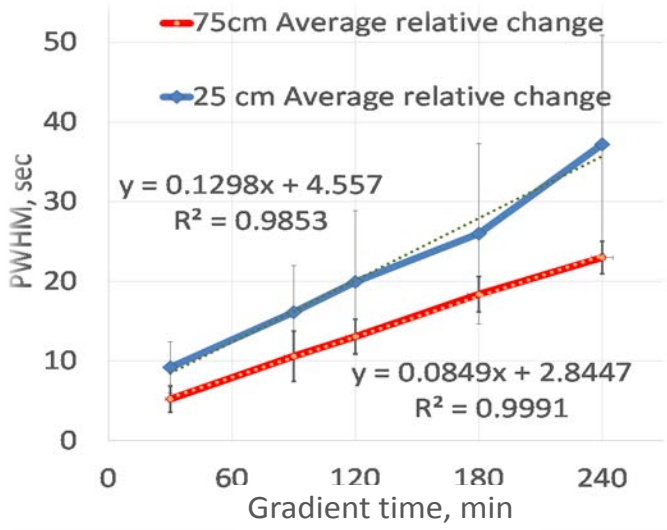
ThermoFisher SCIENTIFIC

Why chromatography is important





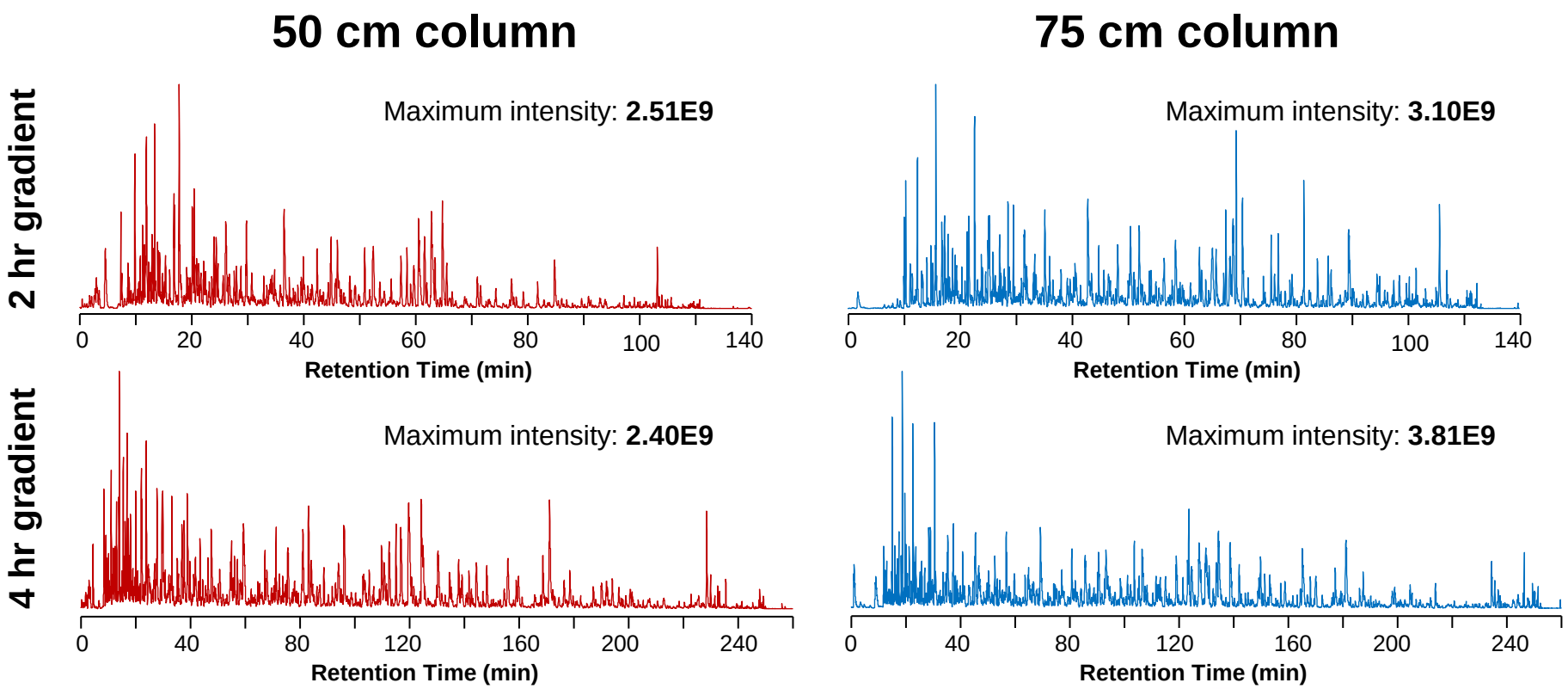
- ✓ Longer gradients give more peak capacity
- ✓ The peak width increases proportionally to the gradient time
- ✓ Longer columns should be used with long gradients



Thermo Scientific™ EASY™-spray, ES805 and ES802

ThermoFisher SCIENTIFIC

Increased protein identification rates with 75 cm columns



- Comparison of 75 cm and 50 cm EASY-Spray column
  - Sample: HeLa Digest (1 µg)
  - Gradient: 120 min or 240 min

Application Note **AN639**

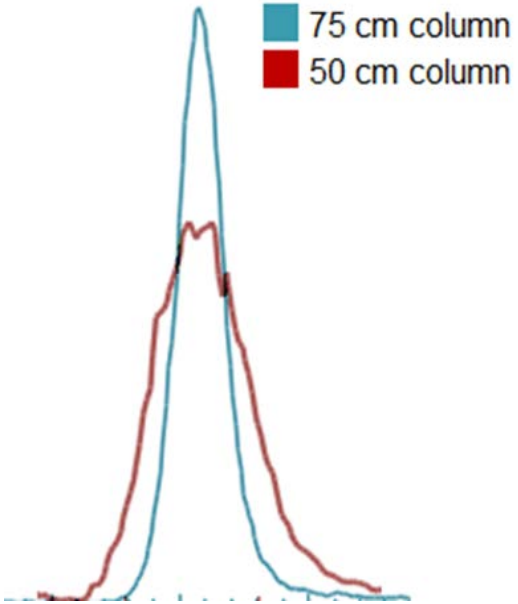
Thermo Scientific™ EASY-nLC™ 1200 system

Thermo Scientific™ Orbitrap Fusion™ Lumos™ Tribrid™ MS

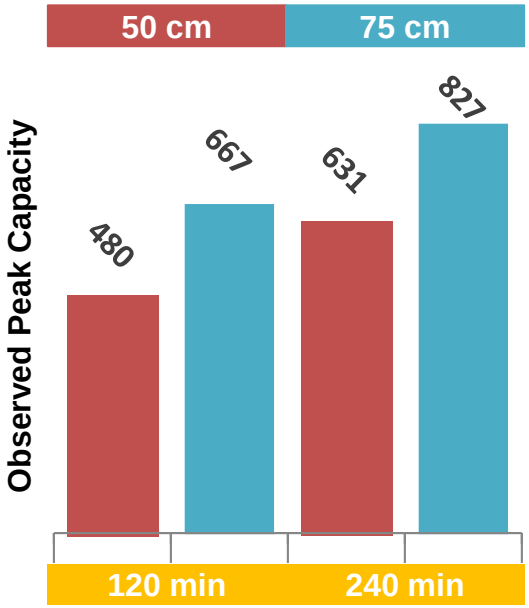
ThermoFisher SCIENTIFIC

Increased protein identification rates with 75 cm columns

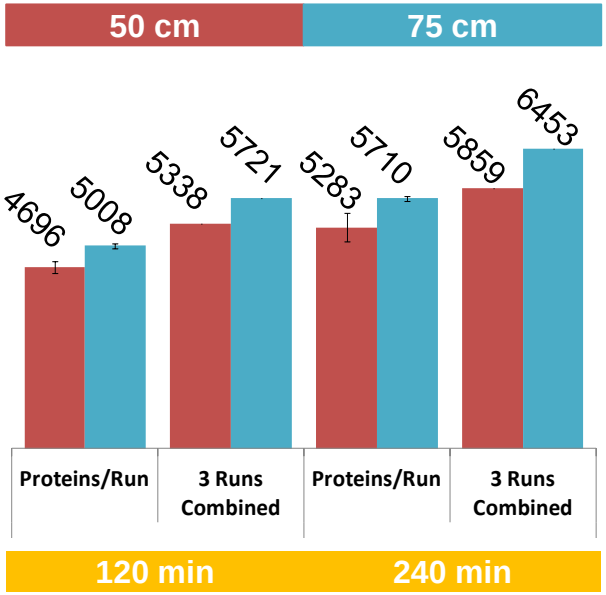
Peak Width



Peak Capacity



Identified Proteins



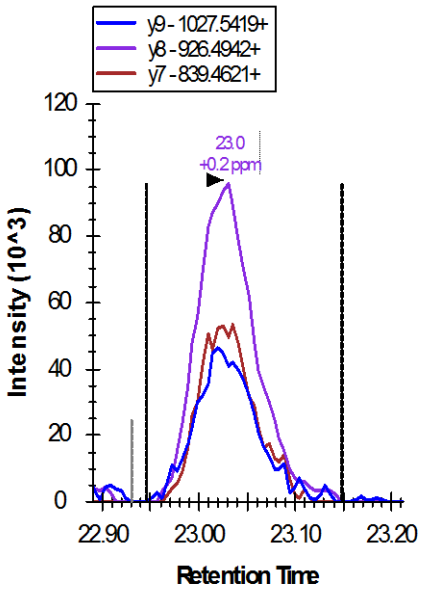
75 cm long columns give:

- ✓ Narrower peak width and higher peaks
- ✓ Higher peak capacity
- ✓ ~ 10% more protein identifications than 50 cm long column

ThermoFisher SCIENTIFIC

NanoLC HRAM MS targeted quantification

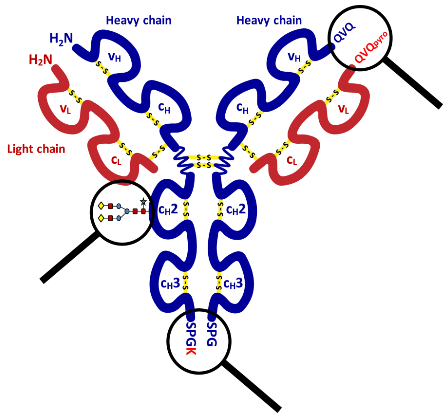
FSGSGSGTSYSLTISR



Inj. Number	Retention time, min
1	23.00
2	23.05
3	23.01
4	23.02
5	23.08
6	23.03
7	23.00
Average	23.03
STD	0.03
RSD, %	0.12

1:10^4 rituximab to HeLa total protein amount in a sample

- 7 consecutive replicates
- 30 min gradient
- EASY-Spray 75 µm x 50 cm, 2 µm
- 4 sec peak width
- 30 min gradient



Multiplexed scheduled HRAM analysis of rituximab in human matrix

Heavy chain: **5 unique peptides**
Light chain: **3 unique peptides**

Thermo Scientific™ UltiMate™ 3000 RSLCnano system

Thermo Scientific™ Q Exactive™ HF hybrid quadrupole-Orbitrap mass spectrometer

✓ LOD at amol level for PRM quantification in matrix
✓ High RT reproducibility permits PRM scheduling

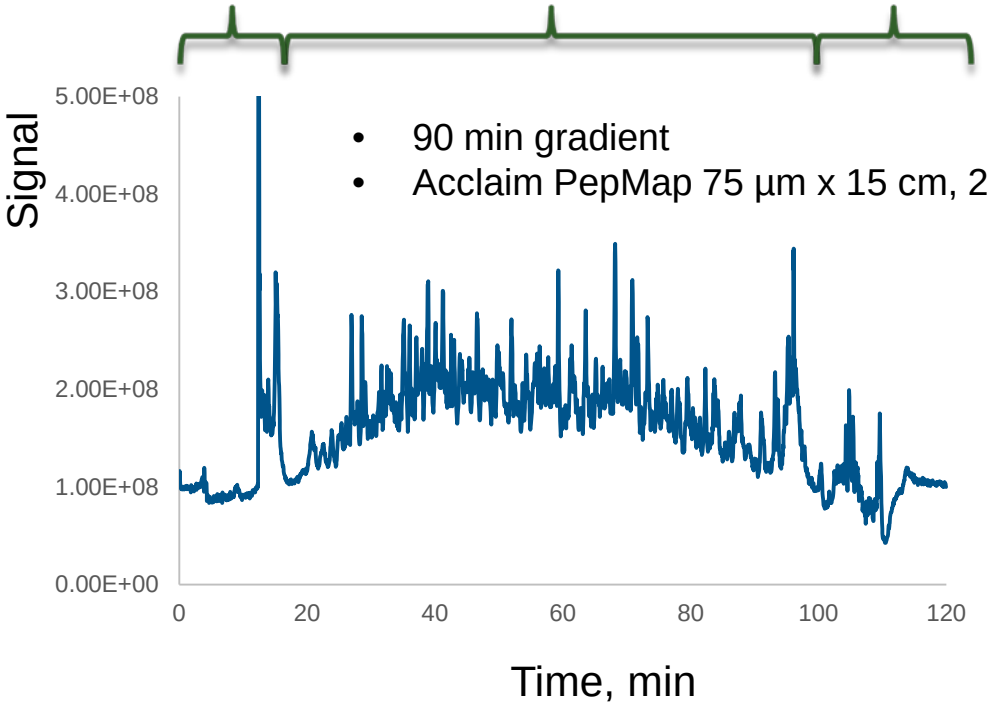
ThermoFisher SCIENTIFIC

Enhanced sensitivity with low nano-flow rates

Sample loading
300 nL/min

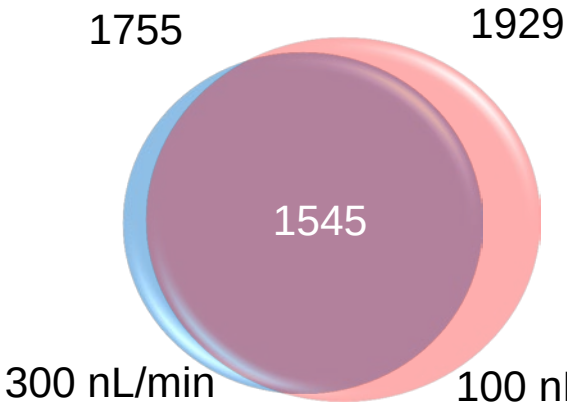
Peptides separation
100, 150, 200 nL/min

Column washing
and re-equilibration
300 nL/min

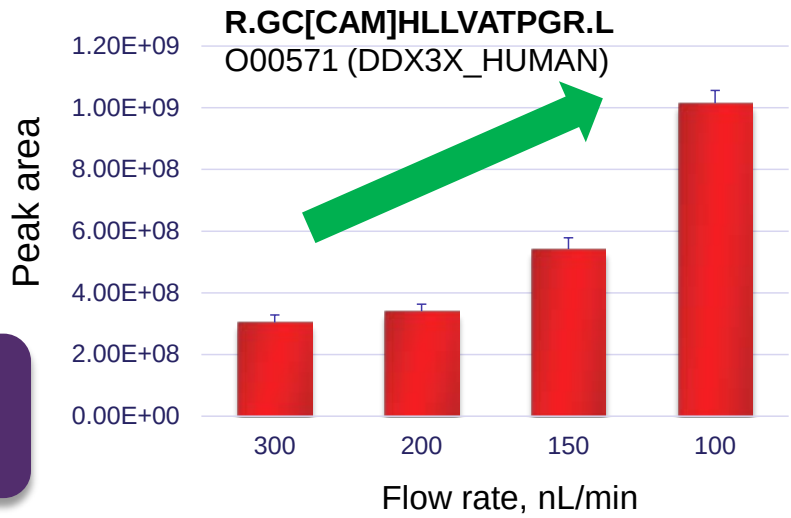


- 90 min gradient
- Acclaim PepMap 75 µm x 15 cm, 2 µm

Protein IDs
20 ng injection



< 1 % false discovery rate (FDR)

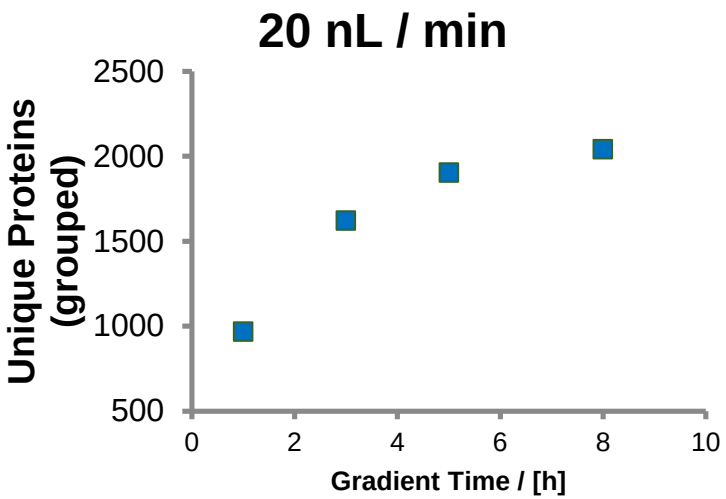


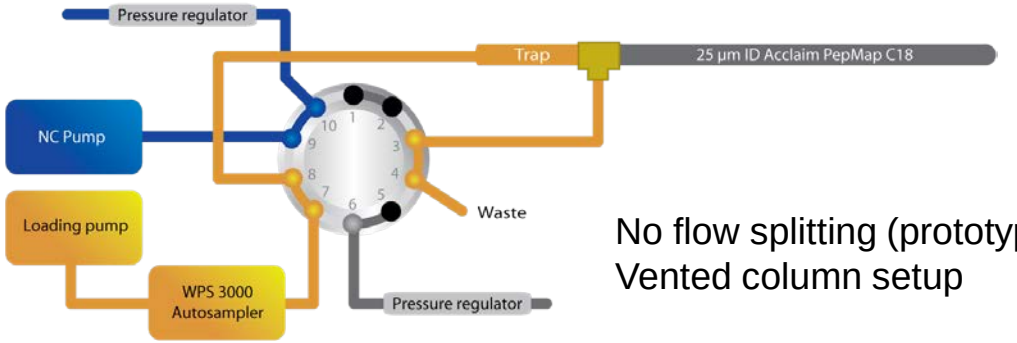
- ✓ More sensitivity for limited sample amounts
- ✓ Same LC-MS setup and gradient length

ThermoFisher SCIENTIFIC

Ultralow-flow nanoLC (100-10 nL/min)

Protein IDs
10 ng injection





No flow splitting (prototype)
Vented column setup

Analytical Column (prototype): 25 µm ID x 25 cm, Acclaim PepMap RSLC C18, 2 µm, 100 Å
Trap: 50 µm ID x 7 cm (2 cm packed), Acclaim PepMap C18, 3 µm, 100 Å
Emitter: 10 µm ID, tip opening 5 µm
Connections: 10 µm ID nanoViper

- ✓ Increased sensitivity in comparison with regular nanoLC-MS
- ✓ Good retention time precision, RSD < 0.2%

Development and performance evaluation of an ultralow-flow nanoliquid chromatography-tandem mass spectrometry set-up; Thomas Köcher et. al., Proteomics, 2014, 14, 1999–2007

ThermoFisher SCIENTIFIC

Conclusions

- Downscaling LC separations results in significant increases in LC-MS sensitivity, permits the analysis of complex samples and facilitates very low level quantification
- The chromatographic scale adopted for an LC-MS method should be chosen according to the relative importance of sensitivity and throughput
- Nano LC-MS using long separation columns is now routine in proteomics
- Ready-to-use capillary connections, integrated plug-and-spray consumables and easy-to-operate LC systems have made nano LC-MS accessible to people with limited LC experience
- Extremely high sensitivity can be obtained using ultra-low nano flow rates. However, a great deal of expertise is required for such setups