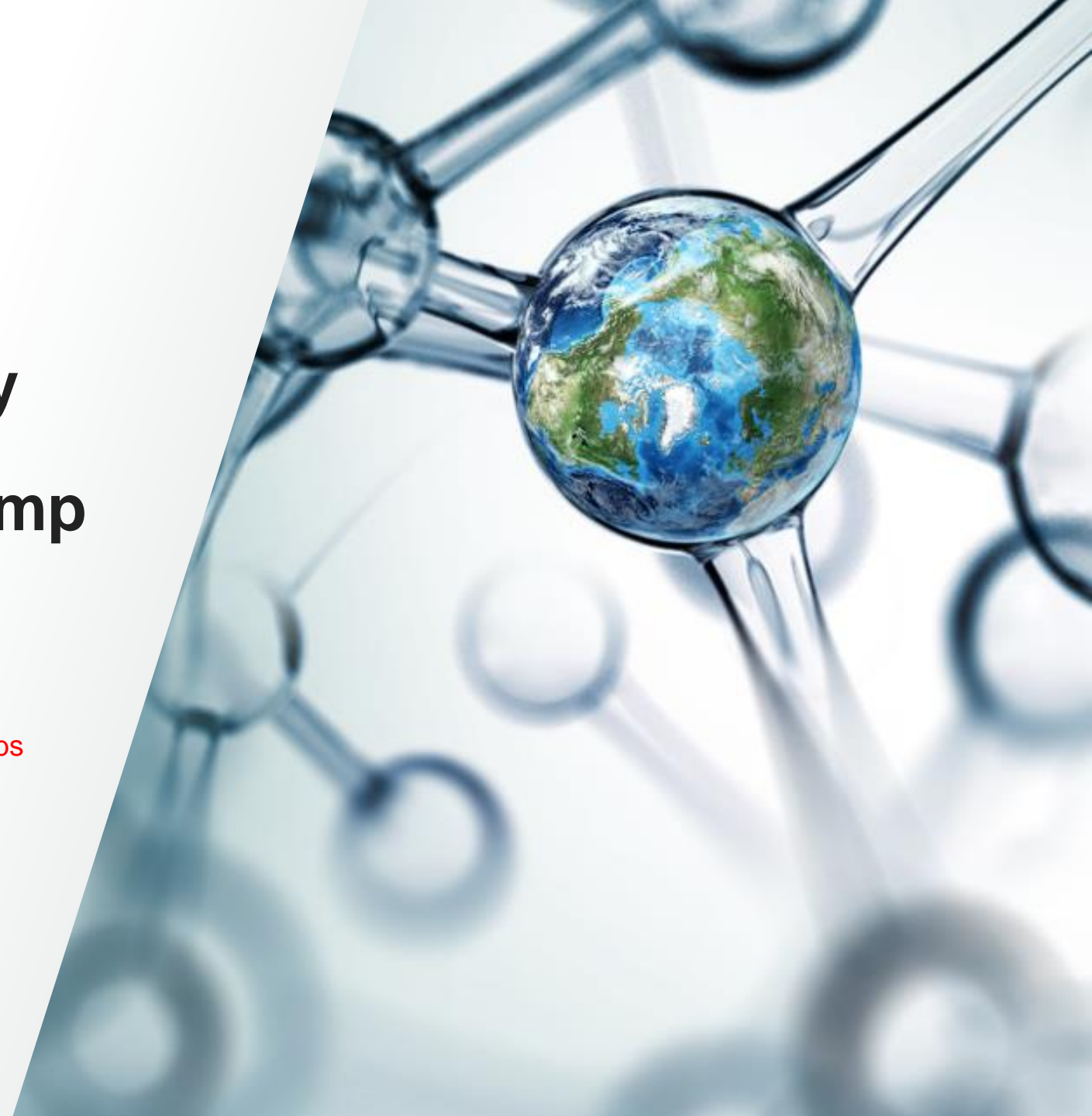


High aspect ratio pillar array columns for deep proteome profiling at moderate LC pump pressures

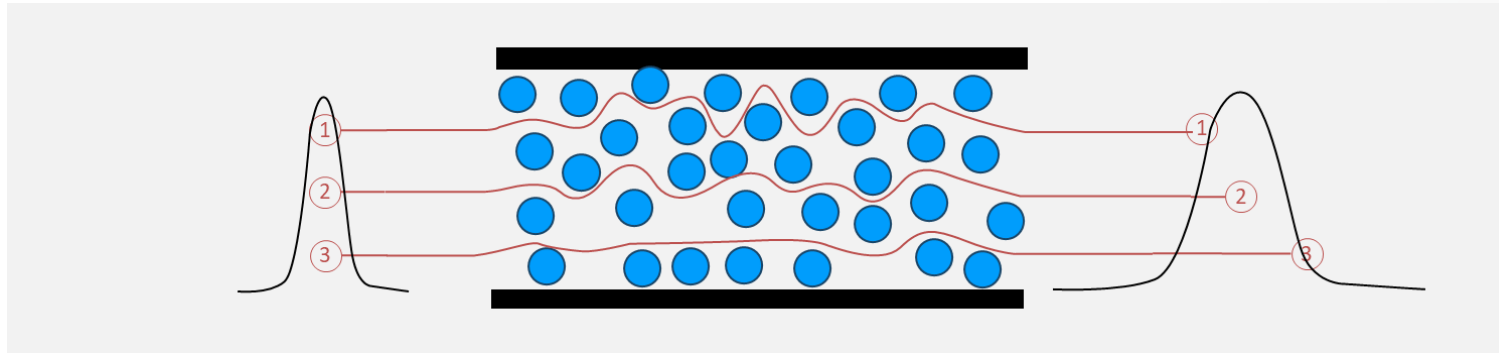
[Jeff Op de Beeck](#), [Natalie Van Landuyt](#), [David Bergen](#), [Romain Huguet](#), [Joshua Silveira](#), [Xuefei Sun](#), [Brandon Robson](#), [Paul Jacobs](#)

 The world leader in serving science

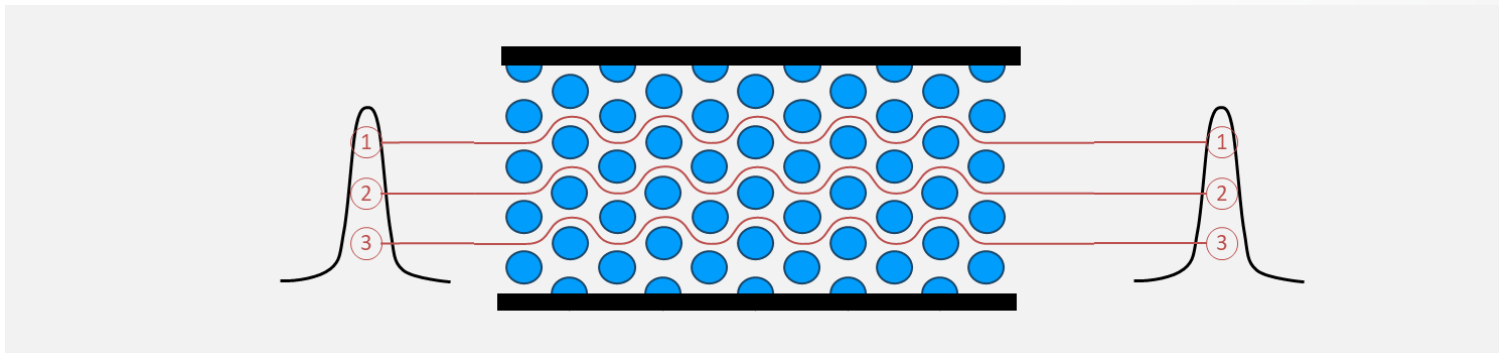
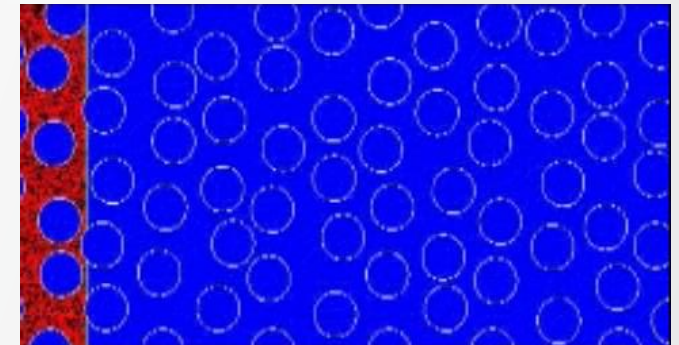


Thermo Scientific™ μ PAC™ HPLC Columns

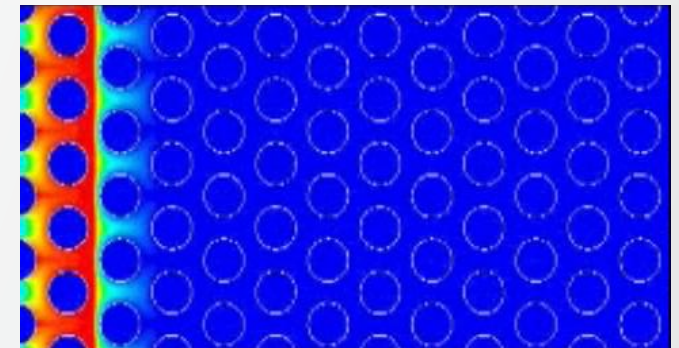
Perfect order in Chromatography



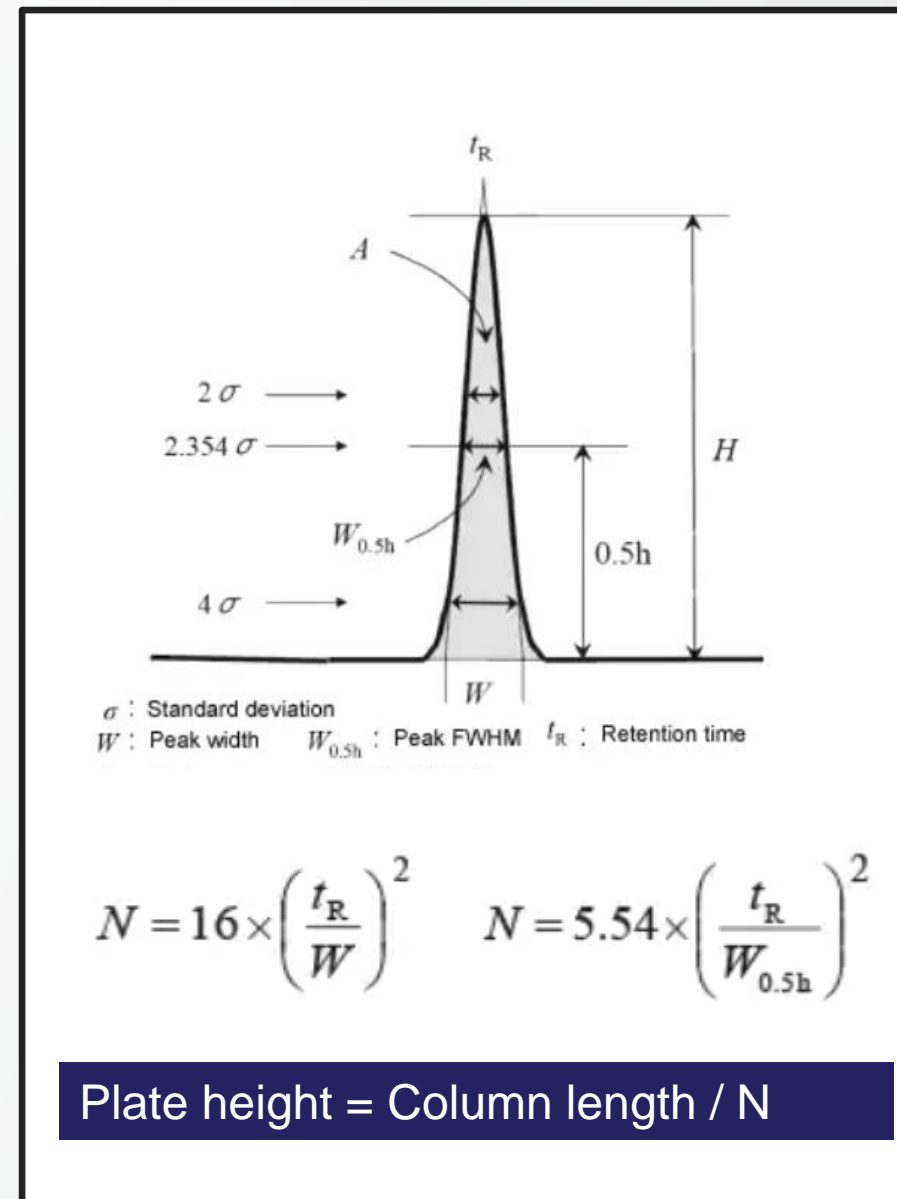
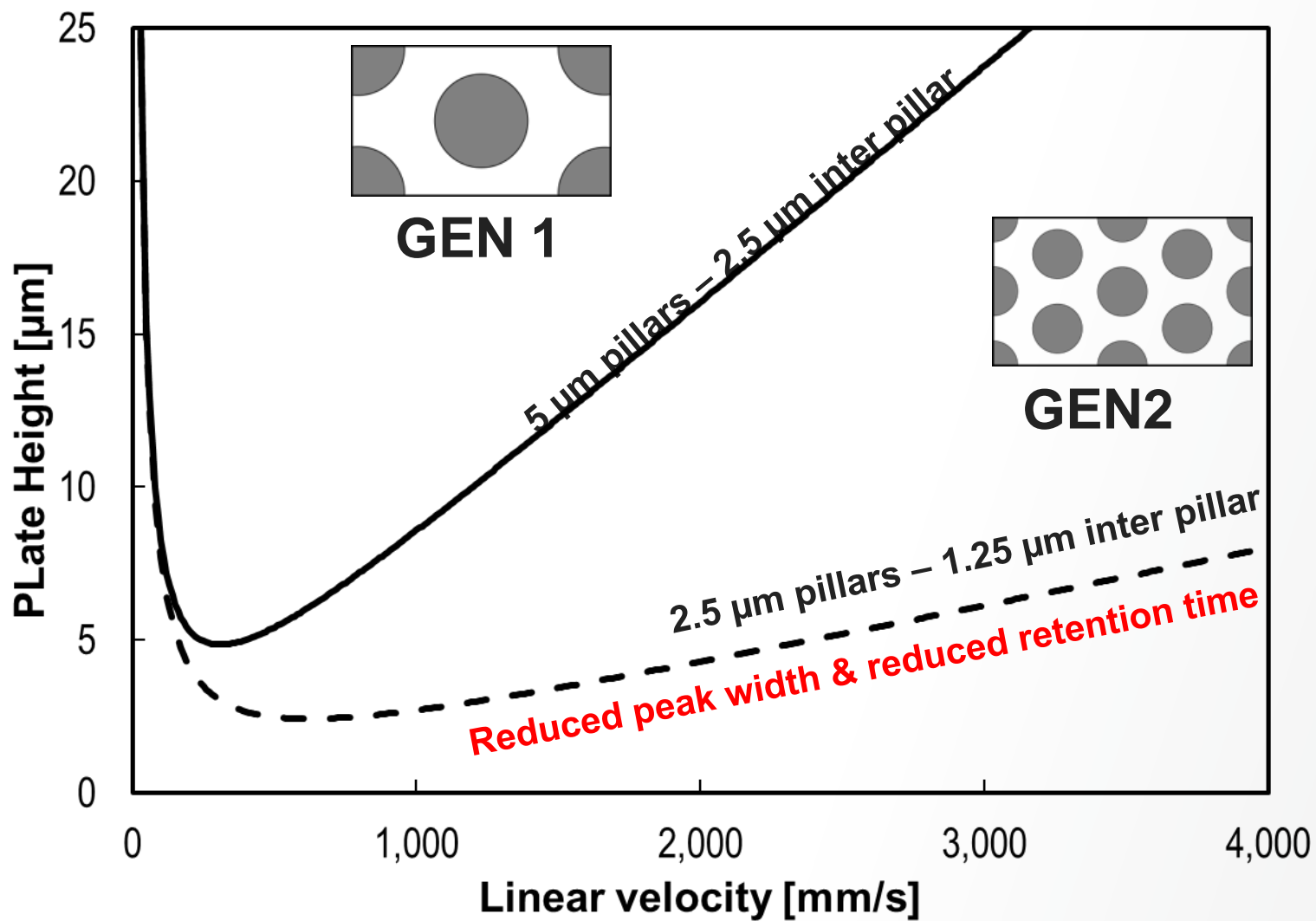
Disorder – Packed bed



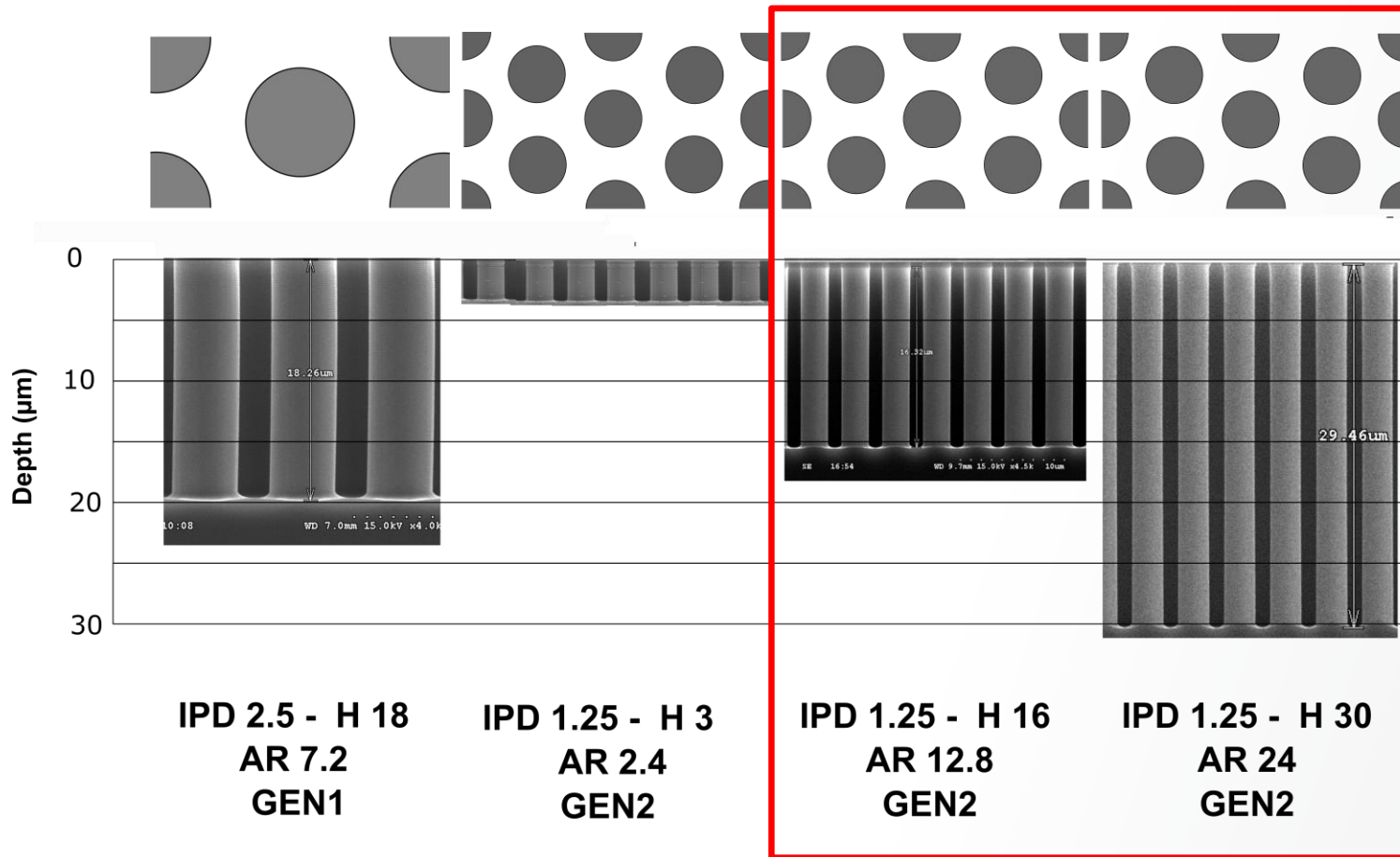
Order – Pillar Array



Effect of downscaling interpillar dimensions



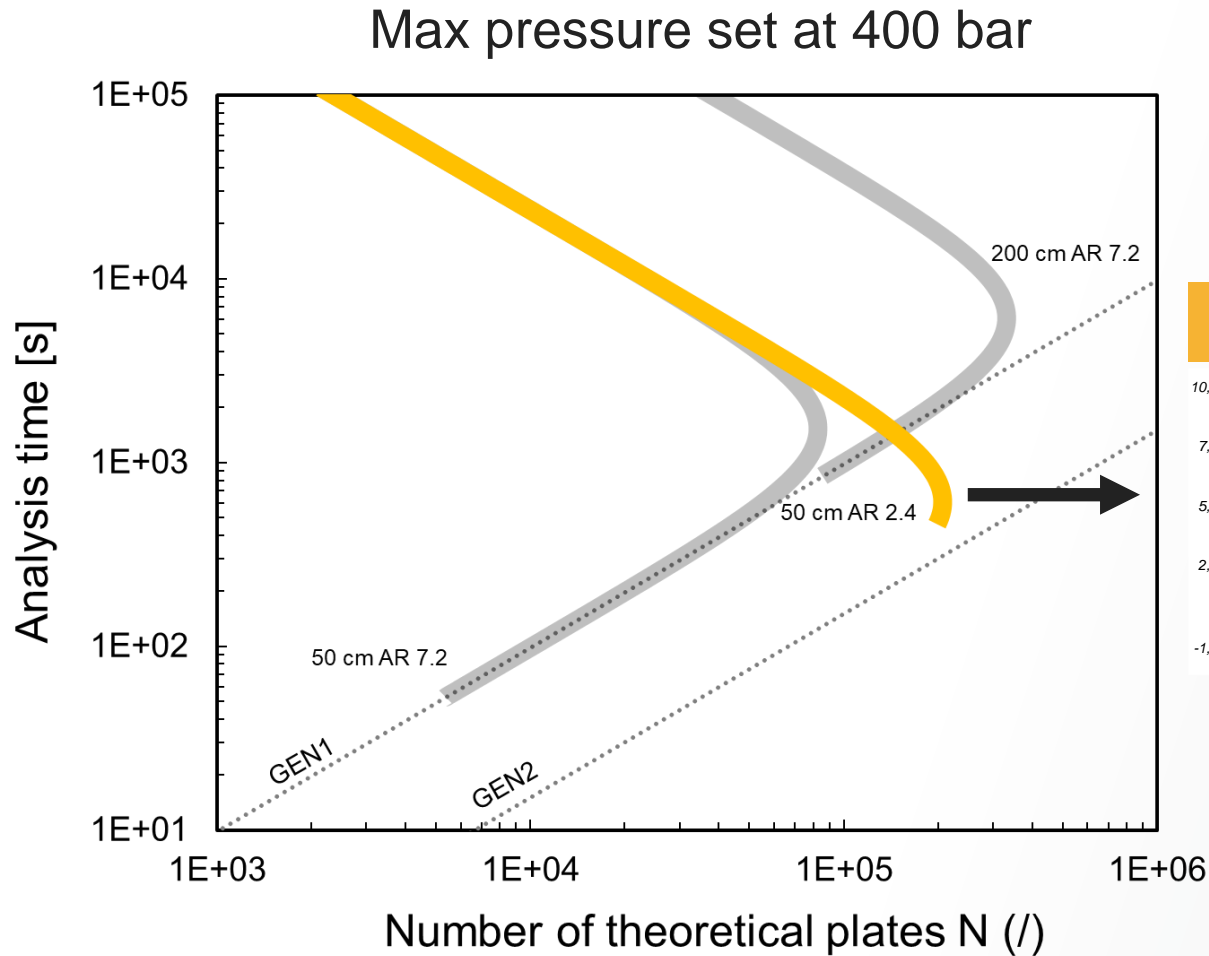
Generation 2 pillar array formats



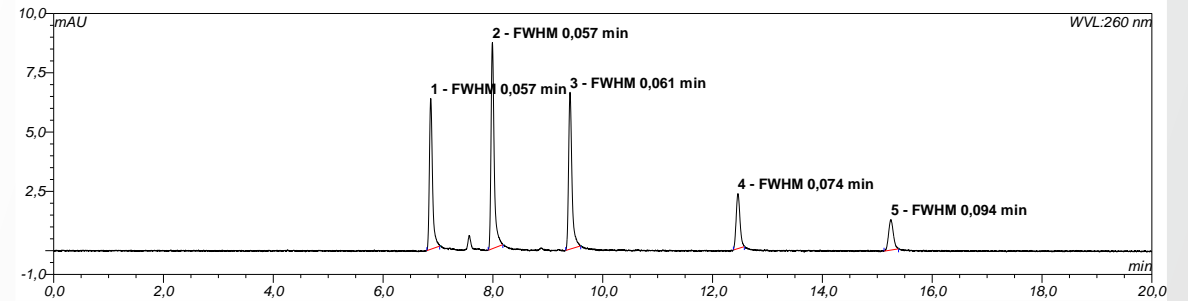
	200 cm GEN1	50 cm GEN2 - 2021	50 cm GEN2 - 2022	110 cm GEN2
Pillar diameter [µm]	5	2.5	2.5	2.5
Inter pillar distance [µm]	2.5	1.25	1.25	1.25
Channel width [µm]	315	1000	180	180
Channel depth [µm]	18	3	16	30
Aspect Ratio [/]	7.2	2.4	12.8	24
Circular eq diameter [µm]	85	60	60	85
Column volume [µL]	9	1.5	1.5	4.5
Max Flow rate [nL/min]	1000	300	750	750

To be Launched as Thermo Fisher™ µPAC™ Neo Column

Kinetic plot – isocratic performance



160K theoretical plates - 15 min – 350 bar

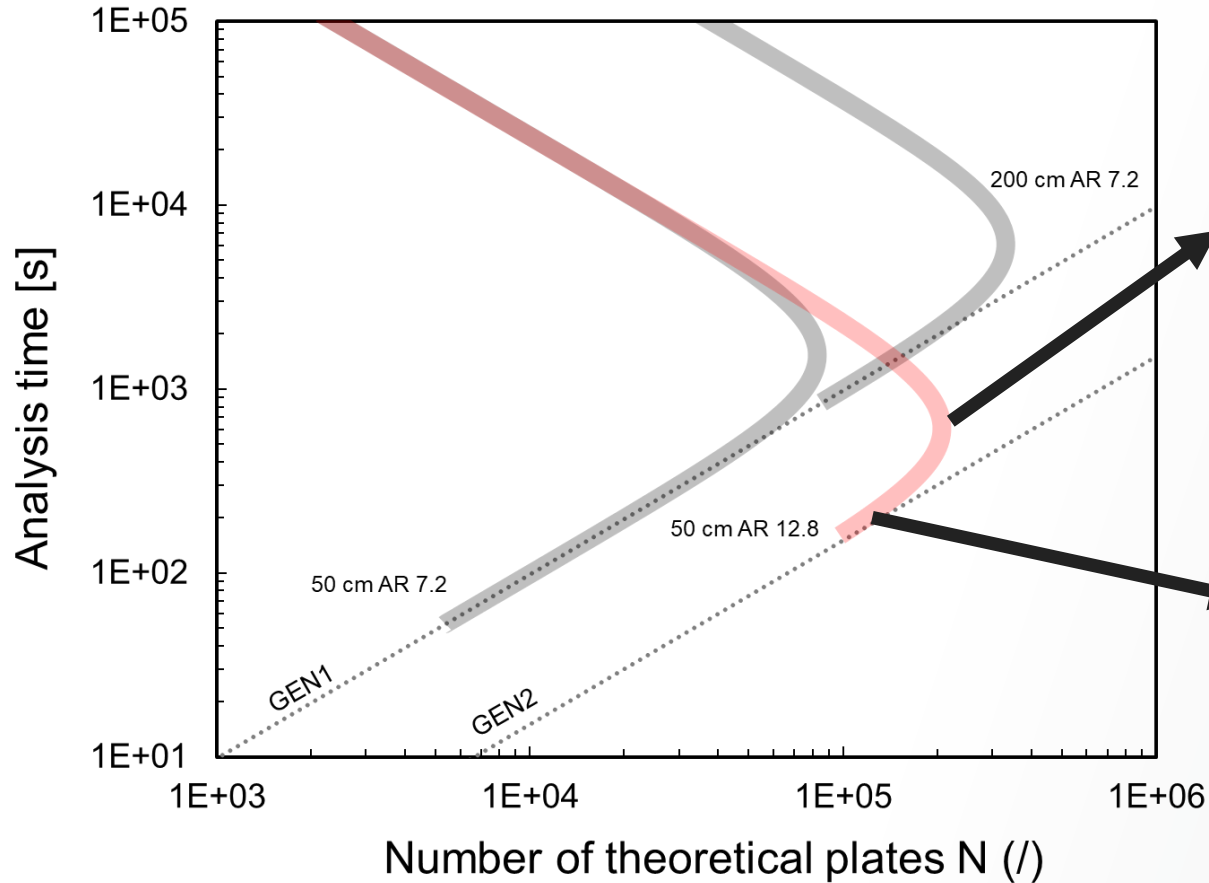


Column: 50 cm AR 2.4 (Thermo Scientific™ μ PAC™ GEN2 HPLC Column prototype 2021)

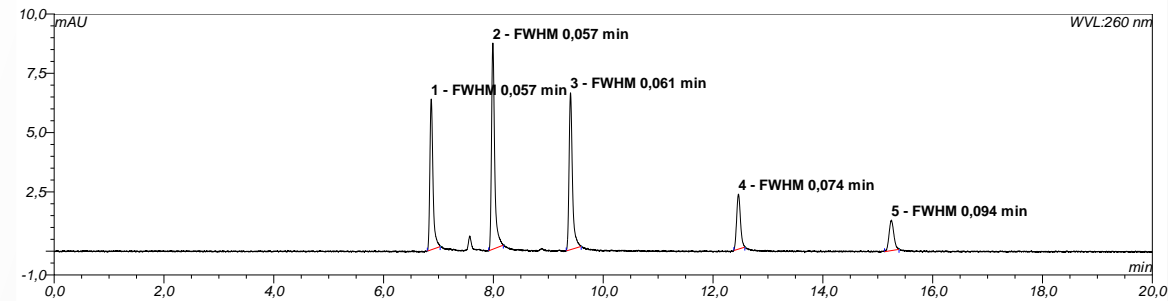
Sample: mixture of Uracil + 4 Alkylphenones, Valerophenone, Propriophenone, Hexanophenone, Octanophenone

Kinetic plot – isocratic performance

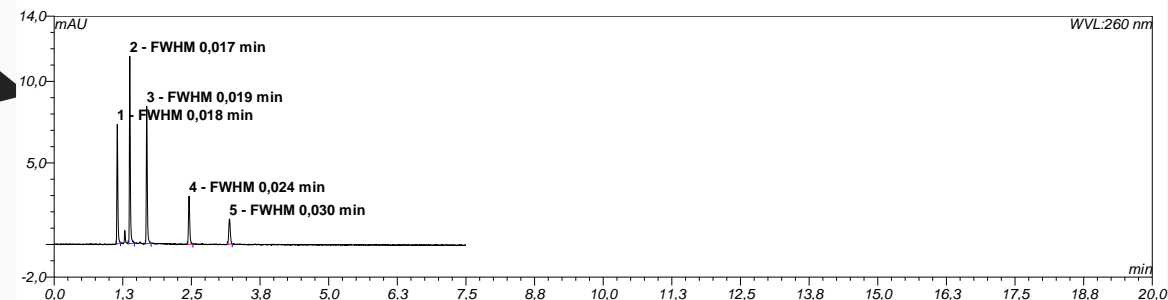
Max pressure set at 400 bar



160K theoretical plates - 15 min – 110 bar



63K theoretical plates – 3.55 min – 350 bar

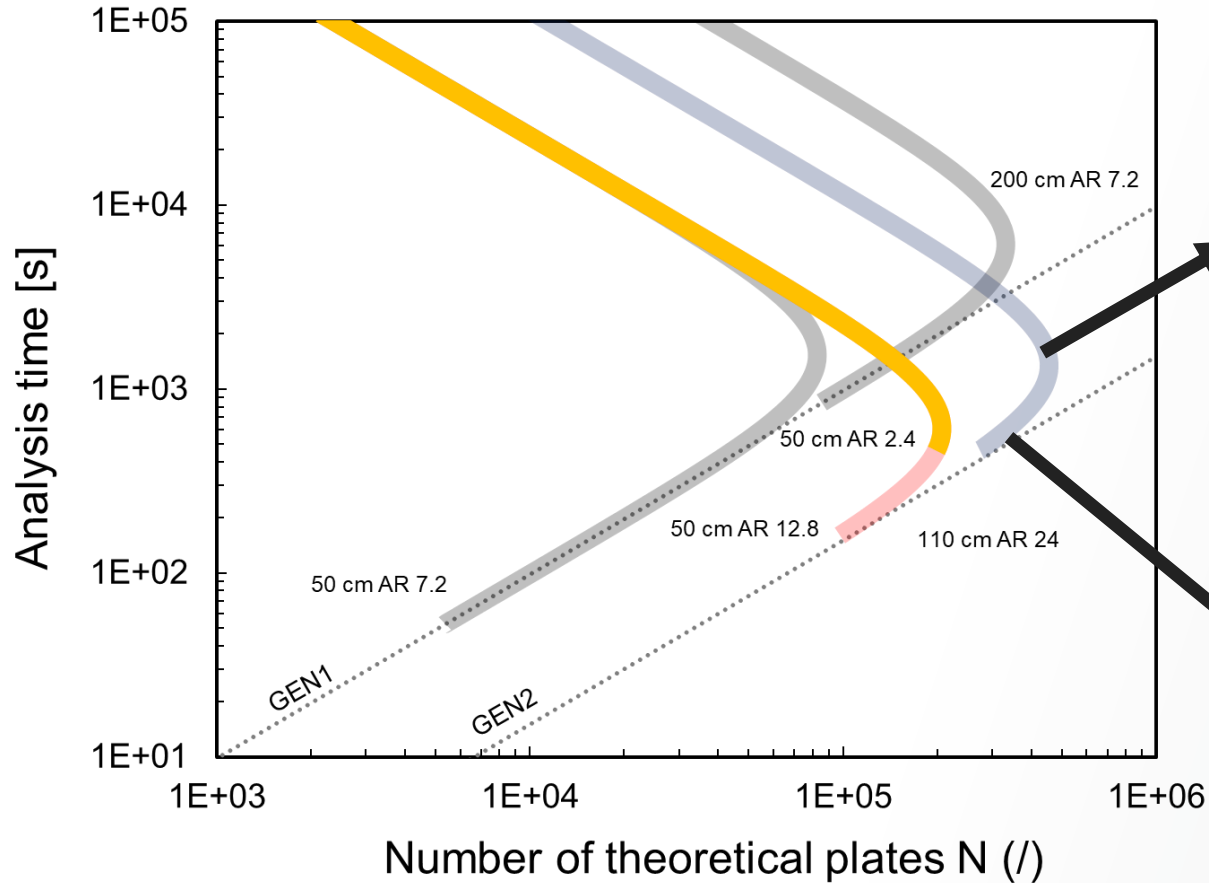


Column: 50 cm AR 12.8 (50 cm μ PAC Neo HPLC Column 2022)

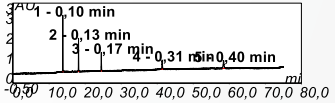
Sample: mixture of Uracil + 4 Alkylphenones, Valerophenone, Propiophenone, Hexanophenone, Octanophenone

Kinetic plot – isocratic performance

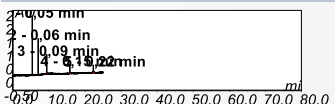
Max pressure set at 400 bar



350K theoretical plates - 60 min – 135 bar



200K theoretical plates – 25 min – 350 bar



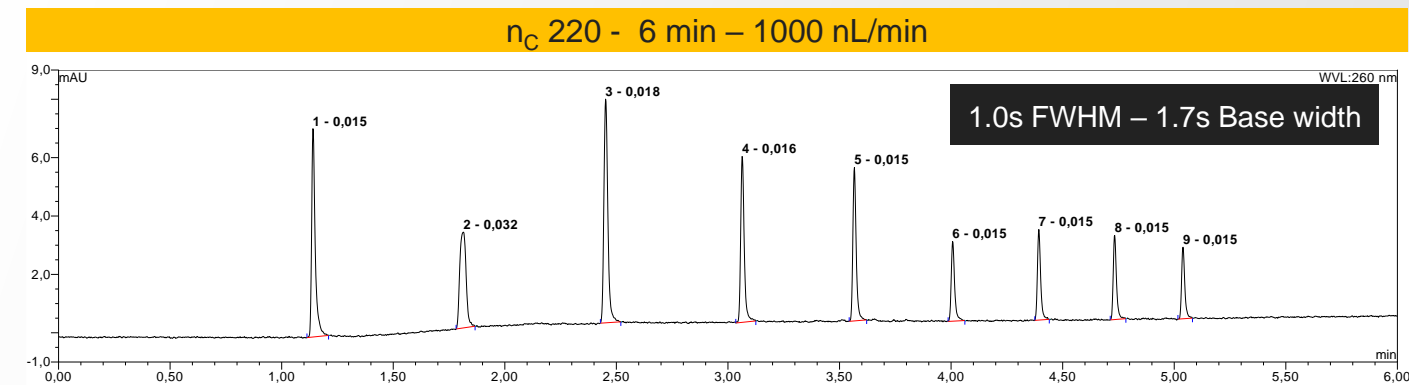
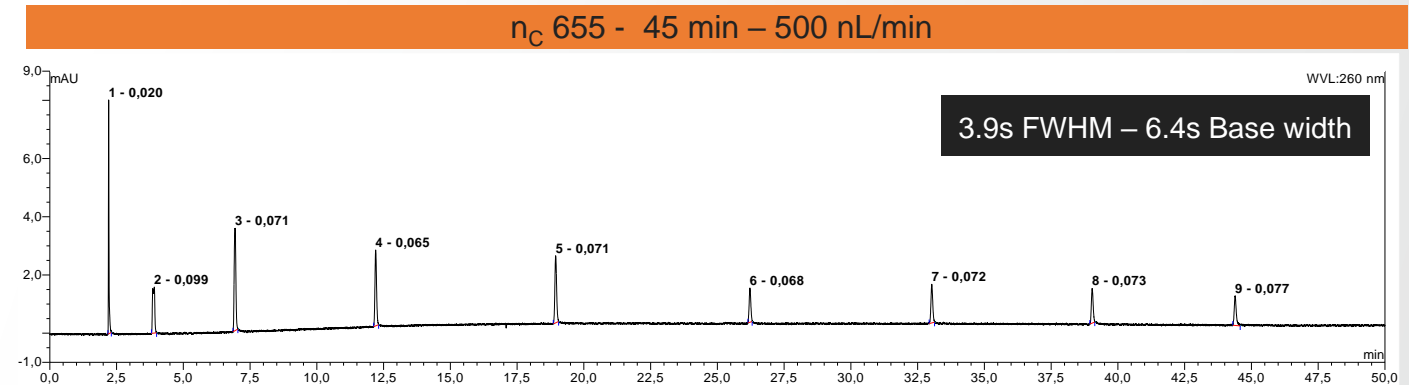
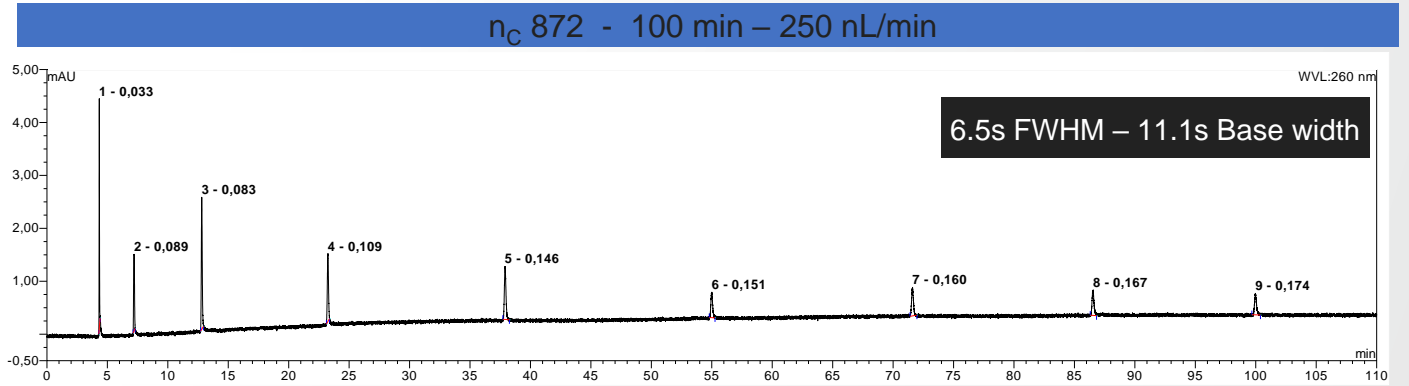
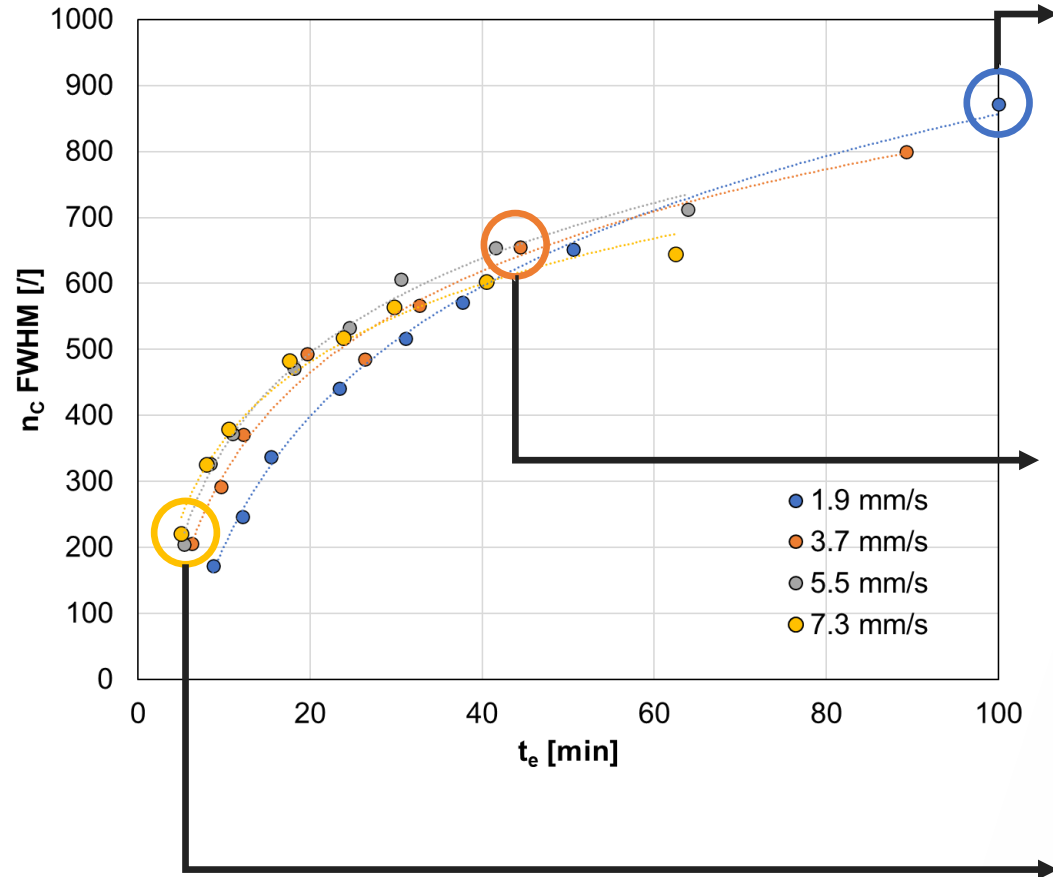
Column: 110 cm AR 24 (110 cm μ PAC Neo HPLC Column prototype 2022)

Sample: mixture of Uracil + 4 Alkylphenones, Valerophenone, Propriophenone, Hexanophenone, Octanophenone

Gradient performance – flow rate comparison

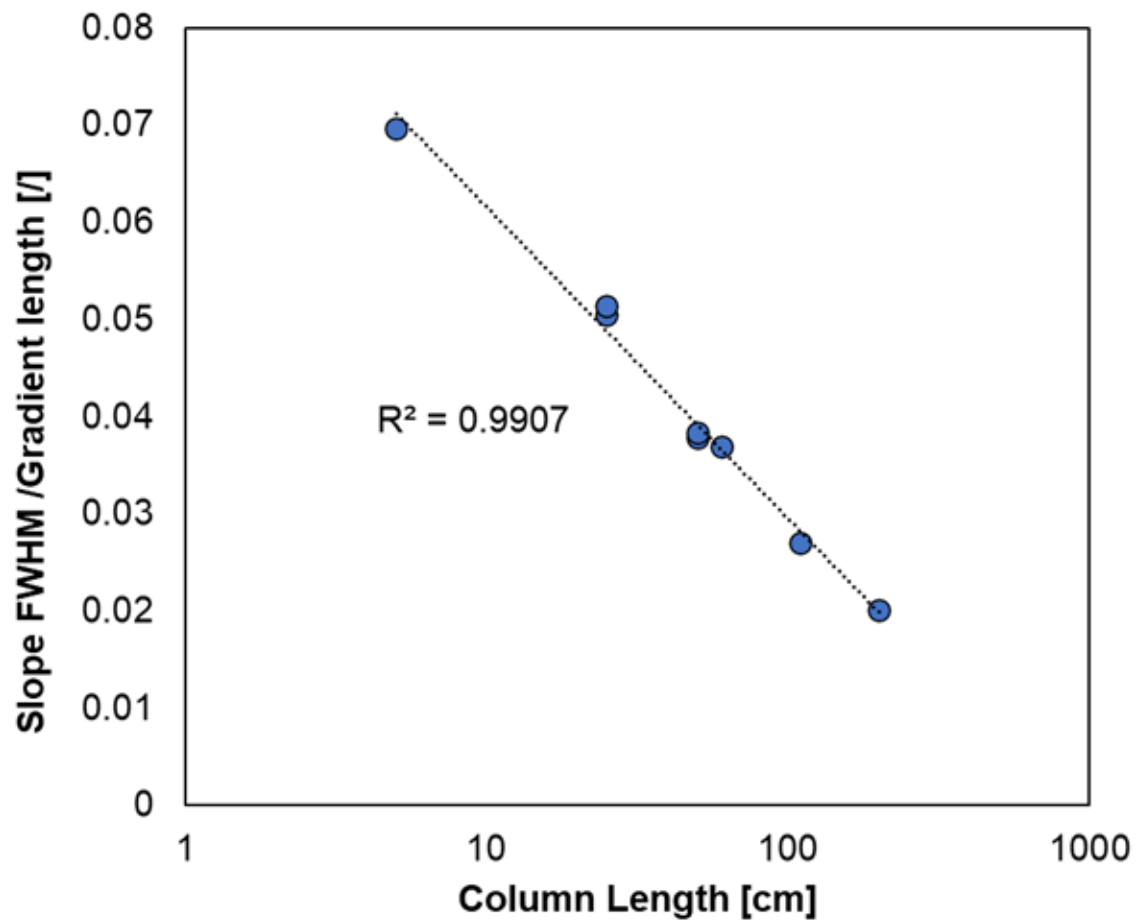
Column: 50 cm AR 12.8 (50 cm μ PAC Neo Column 2022)

Sample: mixture of Uracil + 8 Alkylphenones



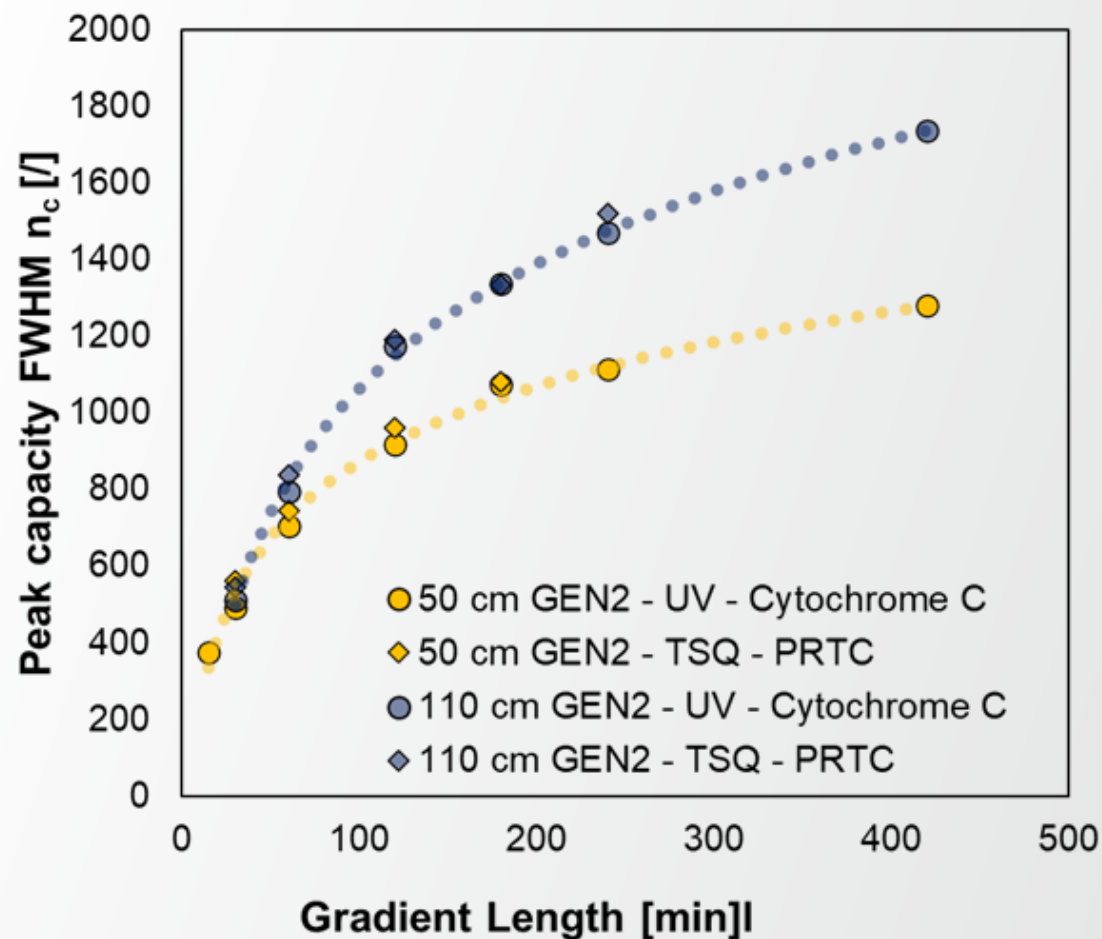
Evolution of peak width – Peak capacity

Peak dispersion rate in gradient elution correlates to column length, irrespective of stationary phase support dimensions

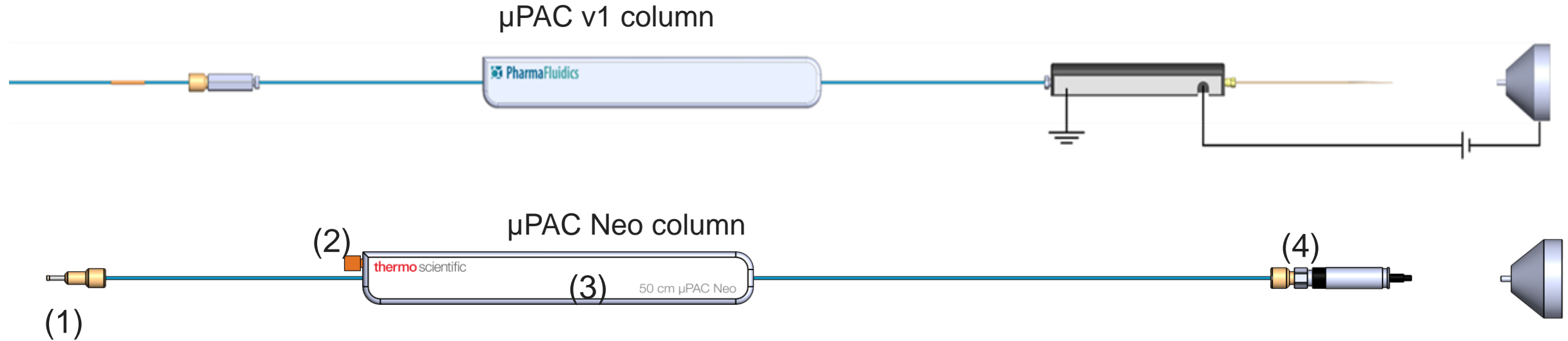


Maximum peak capacity scales with column length

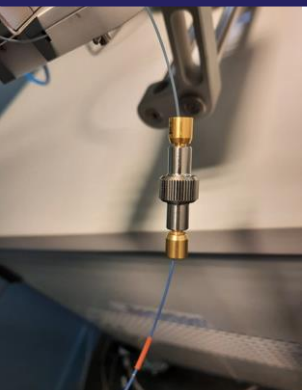
$$n_c \approx \sqrt{L}$$



μPAC Neo column - Ease of use

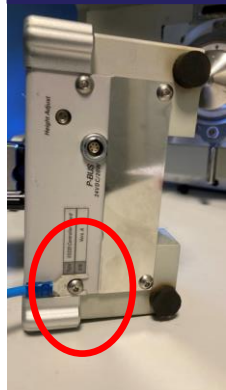


1

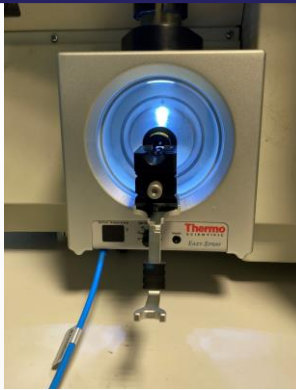


- Column inlet: 280 μm OD / 20 μm ID Thermo Scientific™ nanoViper™
- Thermo Scientific™ Viper™ union (6040.2304)
- Connect with 20μm ID nanoViper coming from injection port

2



- Grounding point on Thermo Scientific™ EASY-Spray™ ionization source
- Needs to be connected with (2) through grounding cable, provided with columns



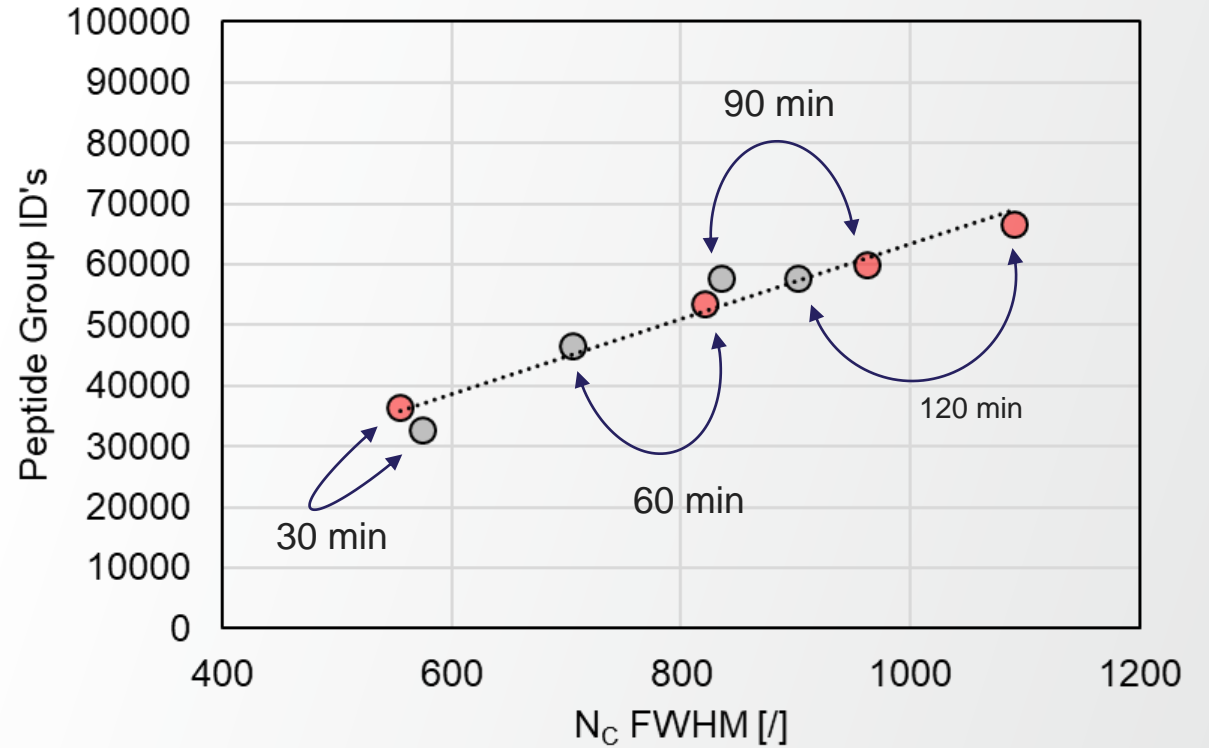
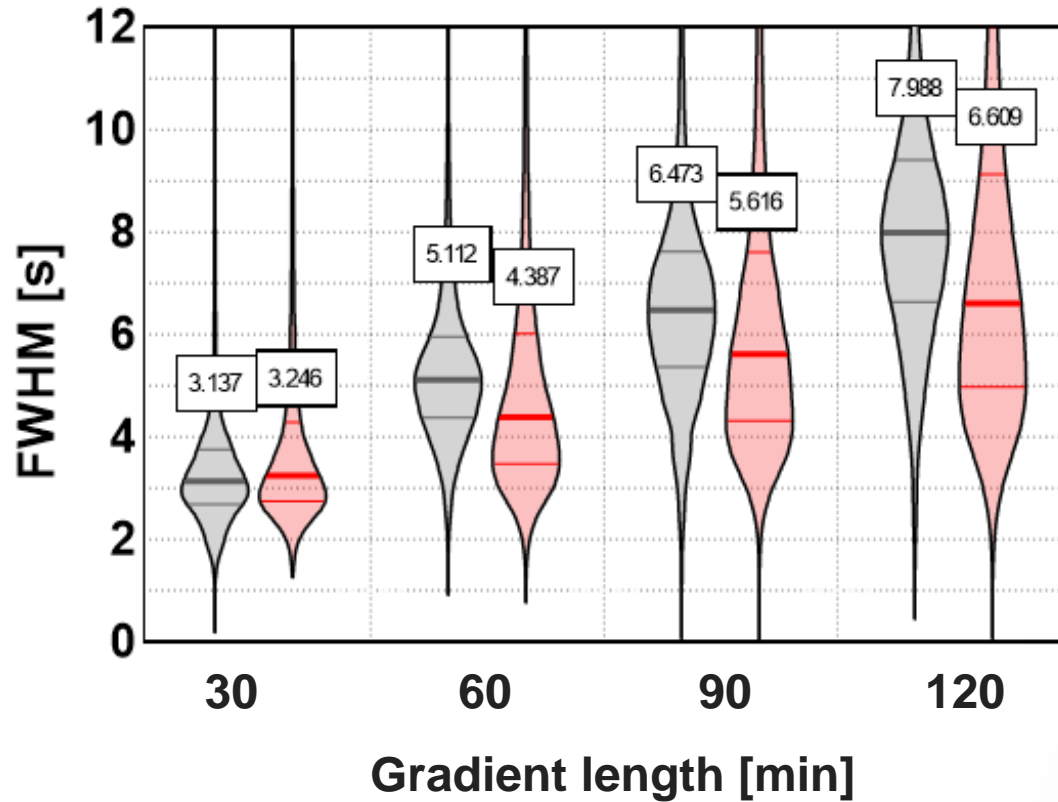
3

- Column casing, preferably placed in LC column compartment
- Alternative or standalone column heating devices also possible

4

- Column outlet: 280 μm OD / 20 μm ID nanoviper
- Connect with ES993 or ES994
- Insert in EasySpray source

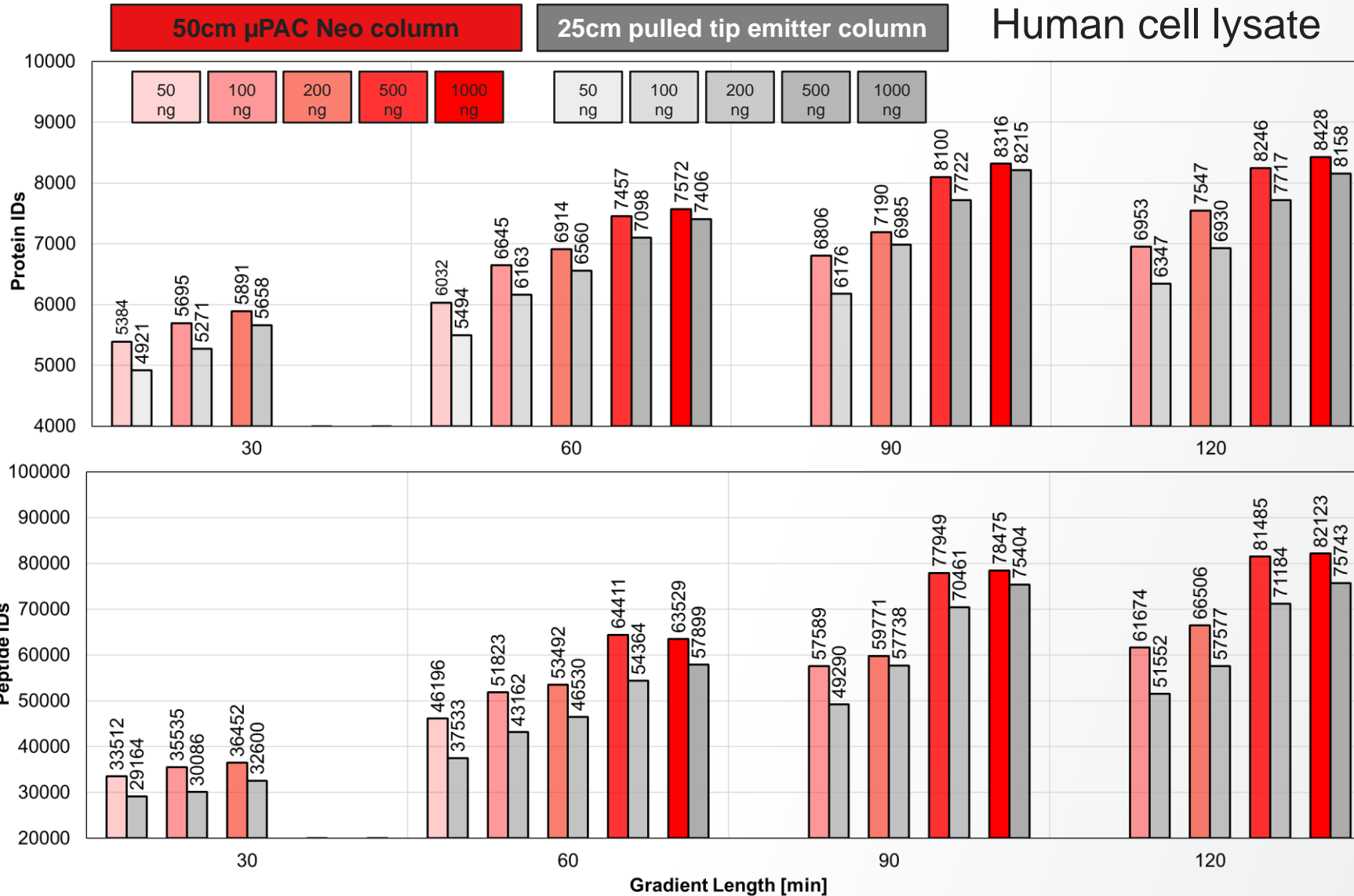
FWHM distribution 200 ng human cell lysate



50cm μ PAC Neo column

25cm pulled tip emitter column

50 cm μ PAC Neo – Bottom-up proteome profiling

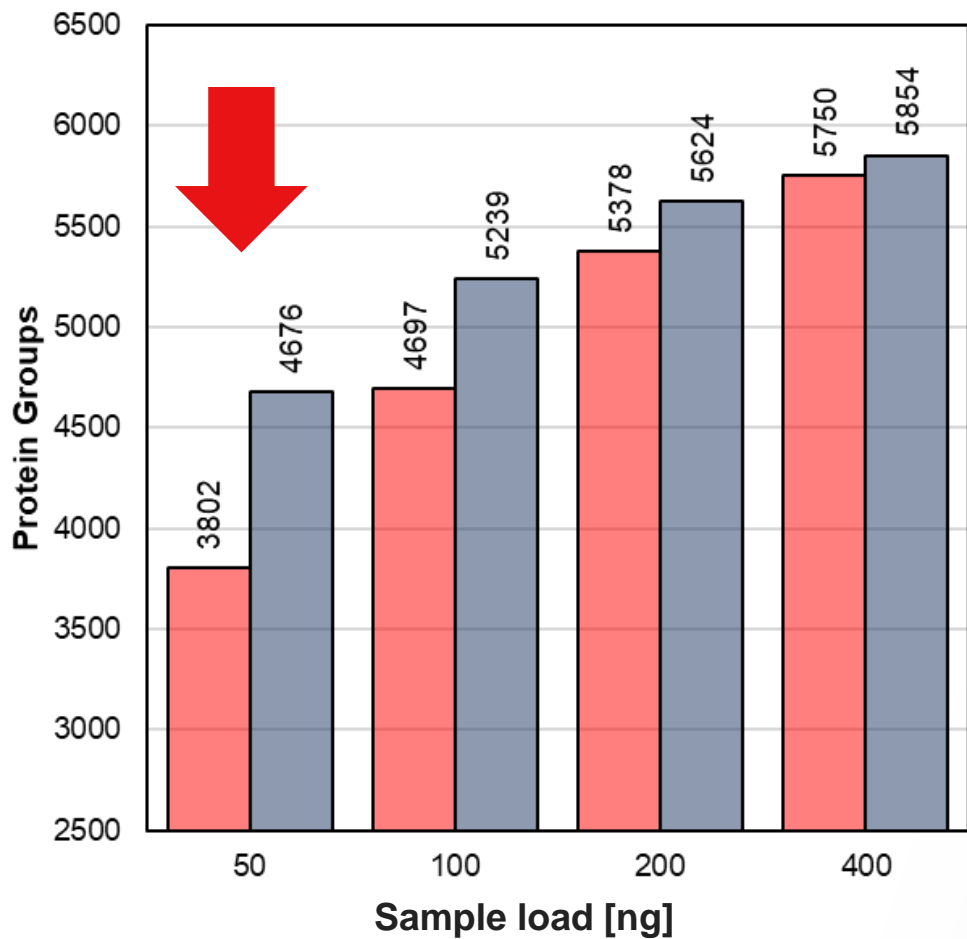


Relative gain

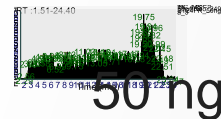
Protein Groups	Gradient [min]	Sample load [ng]				
		50	100	200	500	1000
Protein Groups	30	9%	7%	4%	2%	1%
	60	9%	7%	5%	5%	2%
	90	9%	3%	5%	1%	1%
	120	9%	8%	6%	3%	3%

Peptide Groups	Gradient [min]	Sample load [ng]				
		50	100	200	500	1000
Peptide Groups	30	13%	15%	11%	9%	8%
	60	19%	17%	13%	16%	9%
	90	14%	3%	10%	4%	4%
	120	16%	13%	13%	8%	8%

Combining throughput and sensitivity





Flow rate ramping method 750 to 250 nL/min

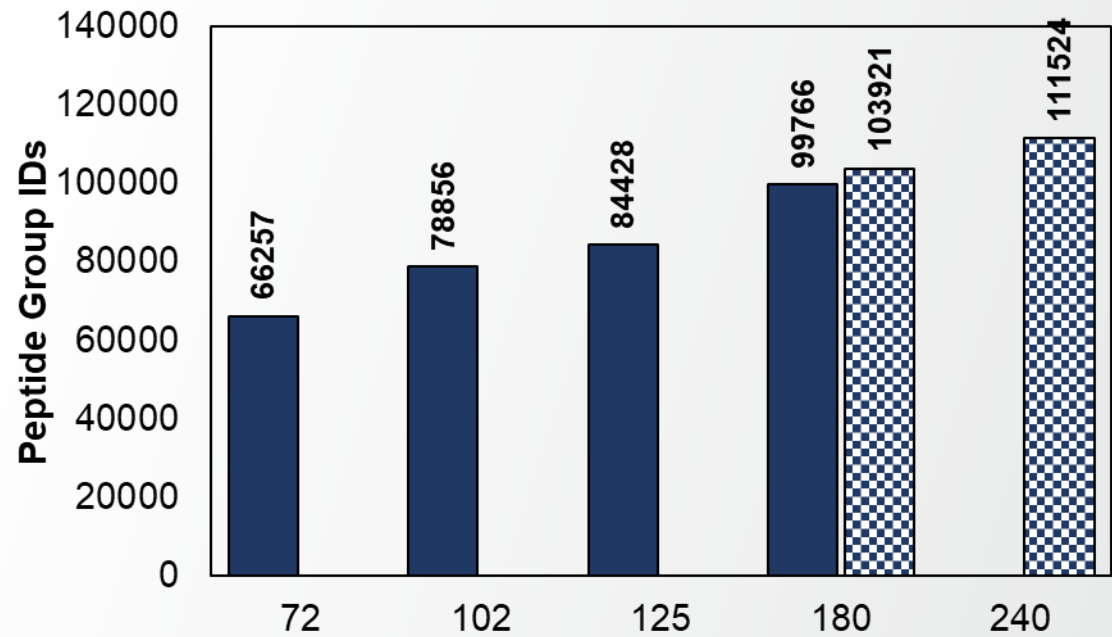
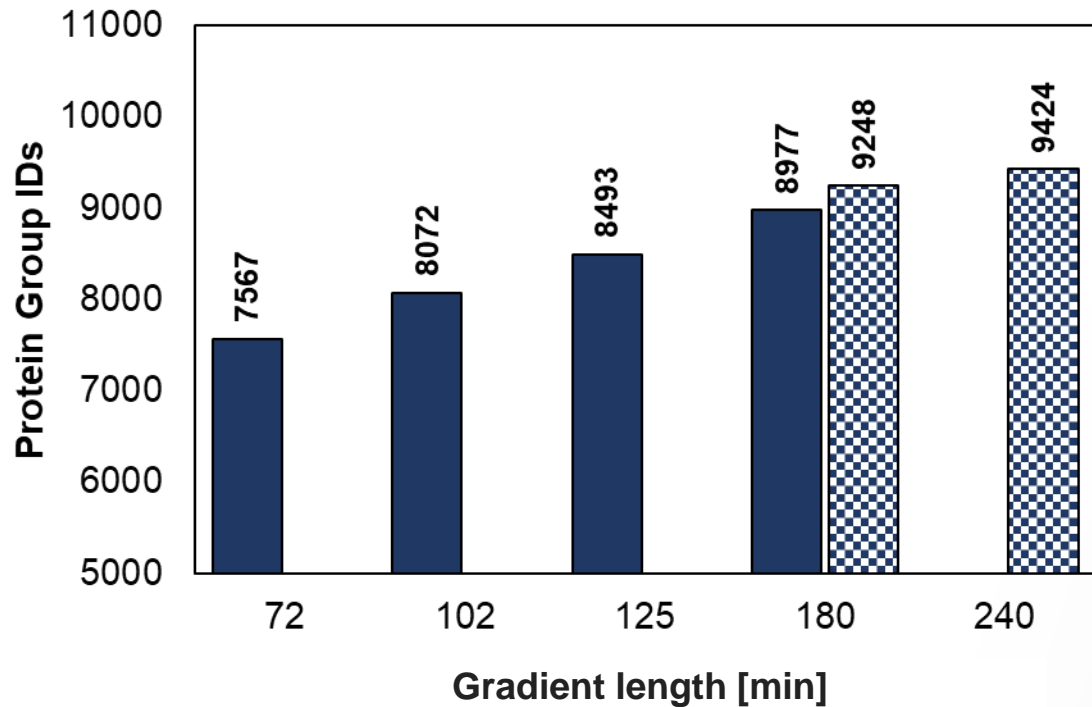


750 nL/min method constant flow



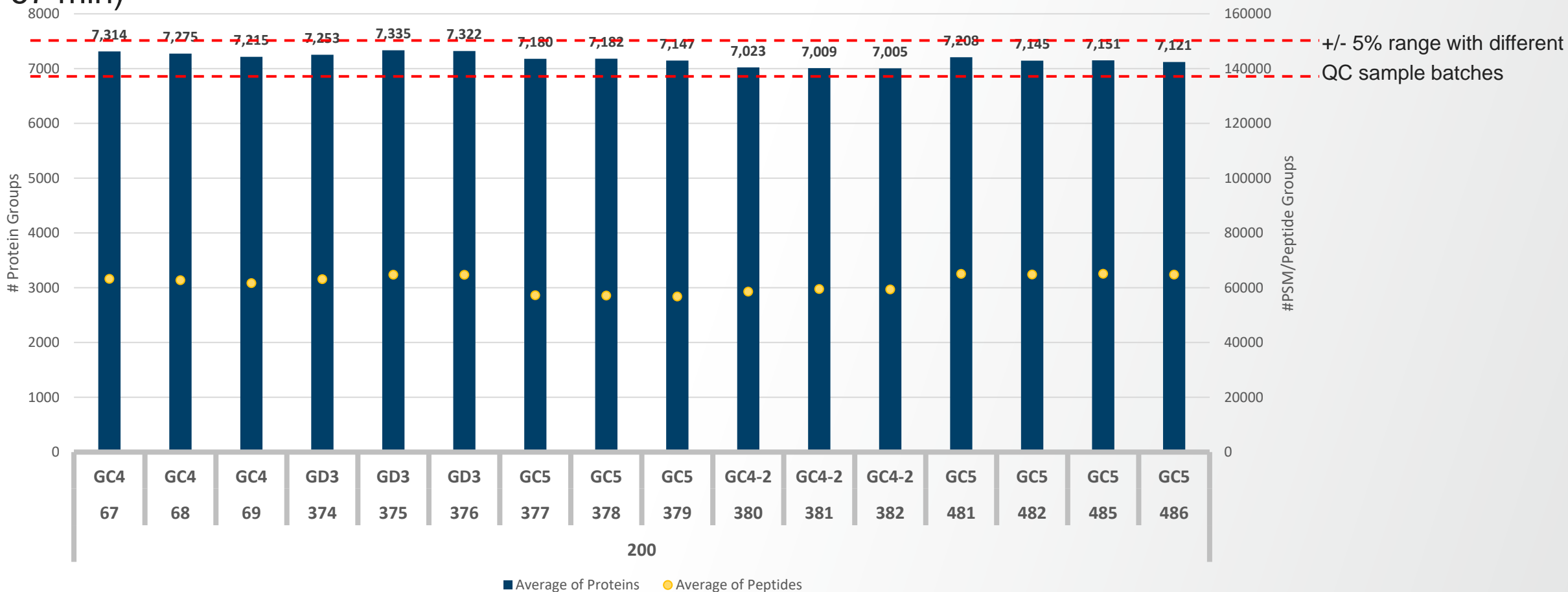
Deep coverage using μ PAC 110 cm

 4 μ g Human cell lysate on column
 1 μ g Human cell lysate on column



50 cm μ PAC Neo column – Long term performance

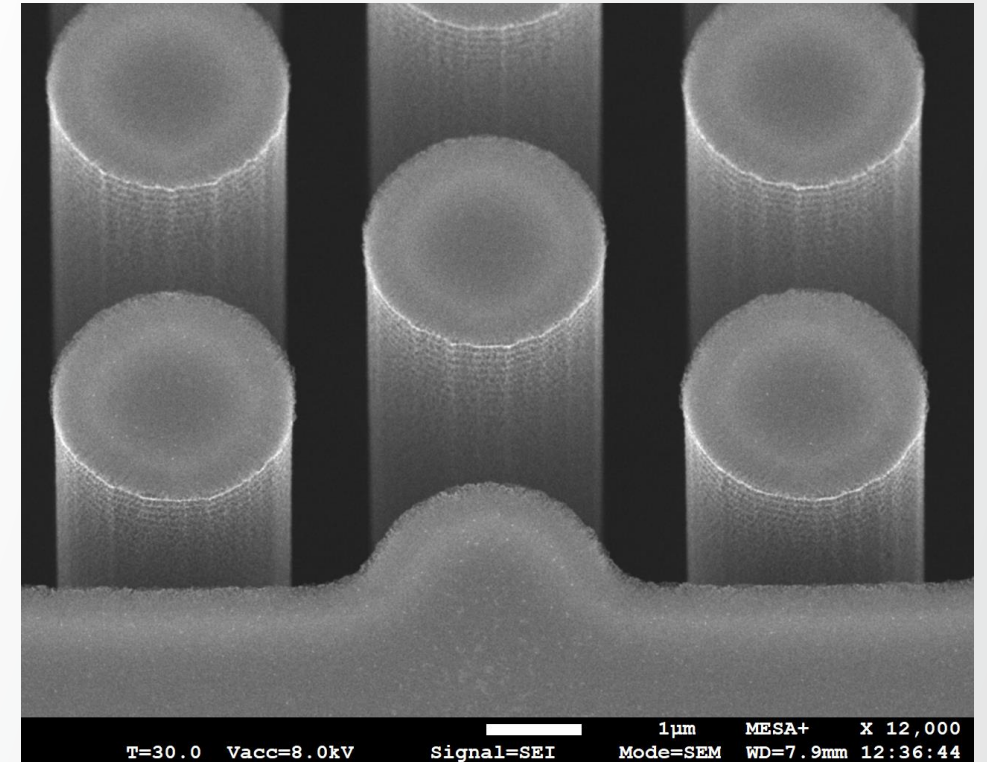
QC sample of 200 ng Human lysate interleaved to quantitative study with hundreds of injections (DDA, 67 min)



Robust & Consistent Performance across 480+ Injections – Single μ PAC Neo HPLC Column

Conclusions

- Downscaling pillar array column support structures results in improved chromatography at reduced analysis times
- Keeping channel cross section constant, but increasing etching depth results in lower operational pressure and higher flow rate flexibility
- New μ PAC Neo format combines improved chromatography, high operational flexibility and fingertight nanoViper easy of use



Questions



