Summary of Dr. Maisch C18 bead testing for LC-MS

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Aims

To assess the quality of peptide chromatography and compare the number of peptide and protein identifications obtained by five different Dr. Maisch C18 beads against the standard 1.9 um ReproSil-Pur 120 C18-AQ beads used at CPR.

C18 beads tested

Name	Size (um)	Part number	Batch
Gold-Turbo ODS-H	1.5	gt115.9h	3813
Platinum C18	1.5	pt115.9e	48/073
Platinum 100 C18 EPS	1.5		66/134
Semi Solid-Core JM PHD	1.5		272
Exsil Pure 120A RP18	1		41/99/3 b
ReproSil-Pur 120 C18-AQ	1.9	r119.aq.0003	

Protocol overview

Approximately 10 mg of each C18 bead type was transferred into a glass vial containing 1 ml of methanol. After the vial was sonicated for 1 minute, home-made 75 um i.d. fused silica columns (360 um o.d.) were packed with the different C18 mixtures (up to 100 bar) in pressure cells until a packed column length of 15 cm was obtained. Columns were then attached to a LC-MS setup (Easy nLC 1200 and a Q Exactive HF) where 500 ng of peptides from tryptically digested HeLa cells were separated over a two hour gradient at a 250 nl/min flow rate. The peptide separation was conducted over a 4% to 20% linear acetonitrile gradient supplemented with 0.1% formic acid. In some situations, gradient conditions (starting flow rate and acetonitrile percentage) were altered in an attempt to improve peptide chromatography. Base peak chromatograms were displayed by Xcalibur software, while protein and peptide identifications were obtained by processing the resulting HeLa raw files with the MaxQuant software suite.

Due to time constraints and machine availability, it was unfortunately not possible to analyse all the bead types in one experiment. When the peptide chromatography performance of each new bead type was investigated, control runs with the standard ReproSil-Pur 120 C18-AQ beads were always performed. This was done to allow fair comparisons to be made and to ensure that any differences between the HeLa peptide stocks used for the experiments were minimised.

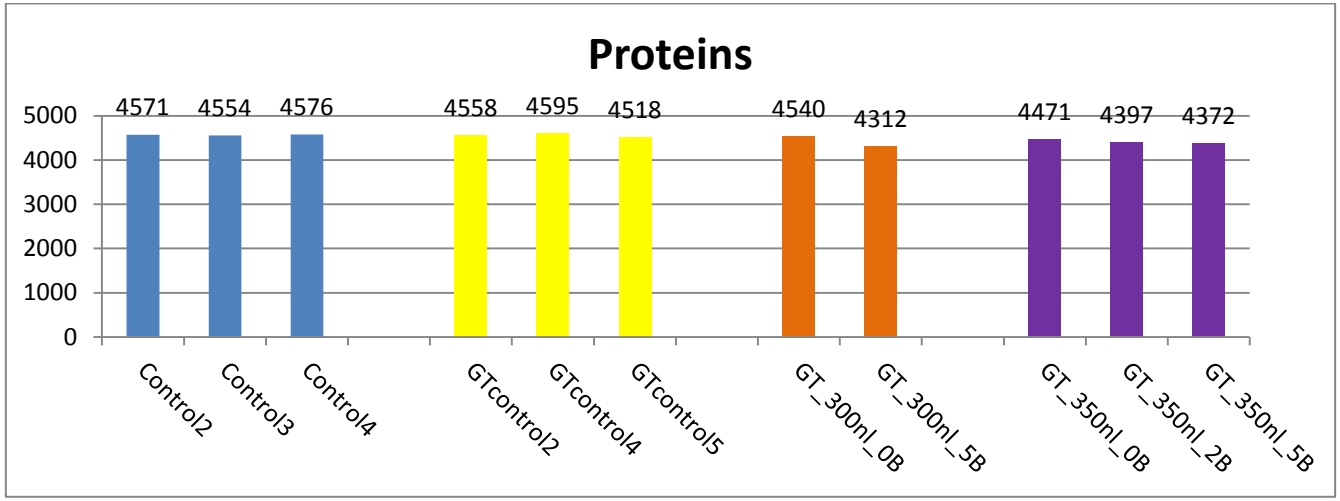
Background of HeLa peptide samples

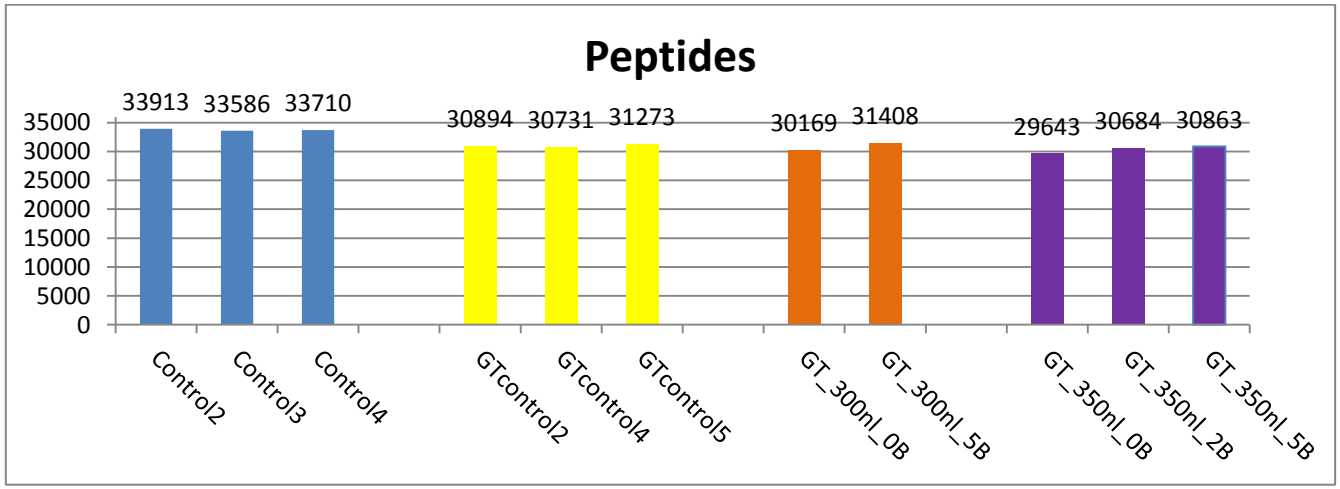
HeLa cells originate from a human cancer cell line and are commonly utilised in cell biology laboratories. When the extracted proteins are digested by the enzyme trypsin, these samples can contain thousands of different peptides with dissimilar physicochemical properties (such as peptide length and overall hydrophobicity), which together provide challenging analytical conditions for the LC-MS setup. They also allow an accurate assessment of the peptide chromatography and mass spectrometer performance that can help determine whether samples from other proteomic experiments are likely to produce useful analytical data.

Results

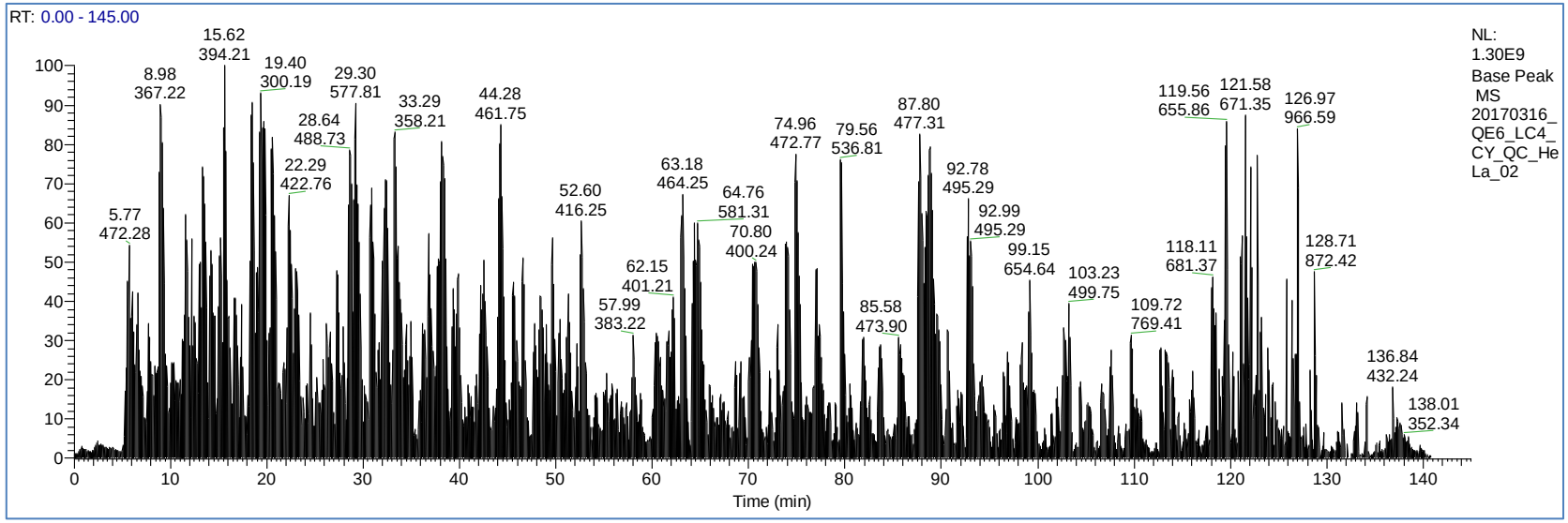
Control 1.9 um ReproSil-Pur 120 C18-AQ beads vs 1.5 um Gold-Turbo ODS-H beads

Compared to the controls (blue bars), the Gold-Turbo C18 runs (yellow) resulted in similar numbers of identified proteins (top graph), but a small loss (10%) with respect to peptides identified (bottom graph). Representative chromatograms of these runs are shown on the next page. Since the chromatography of early eluting peptides was slightly worse in the Gold-Turbo runs, we altered the flow rate and/or the acetonitrile percentage at the start of the gradient (orange/purple) to try and rectify this. Unfortunately, no improvements were observed.

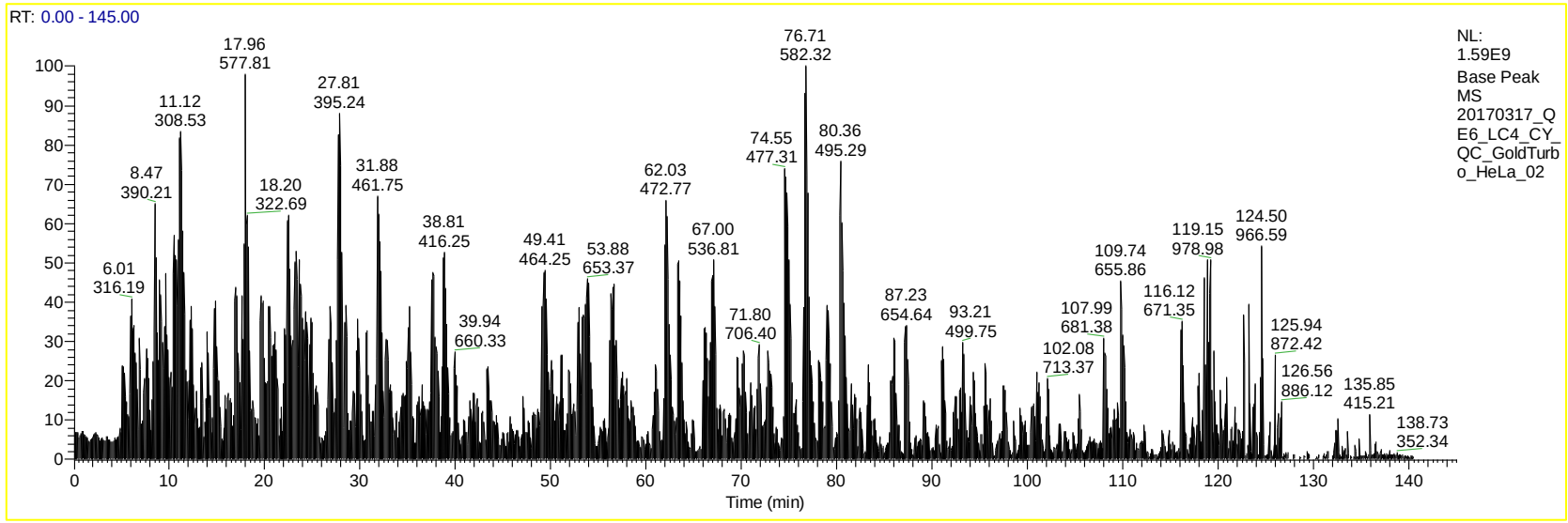




Chromatogram of 1.9 um ReproSil-Pur 120 C18-AQ (Control2)

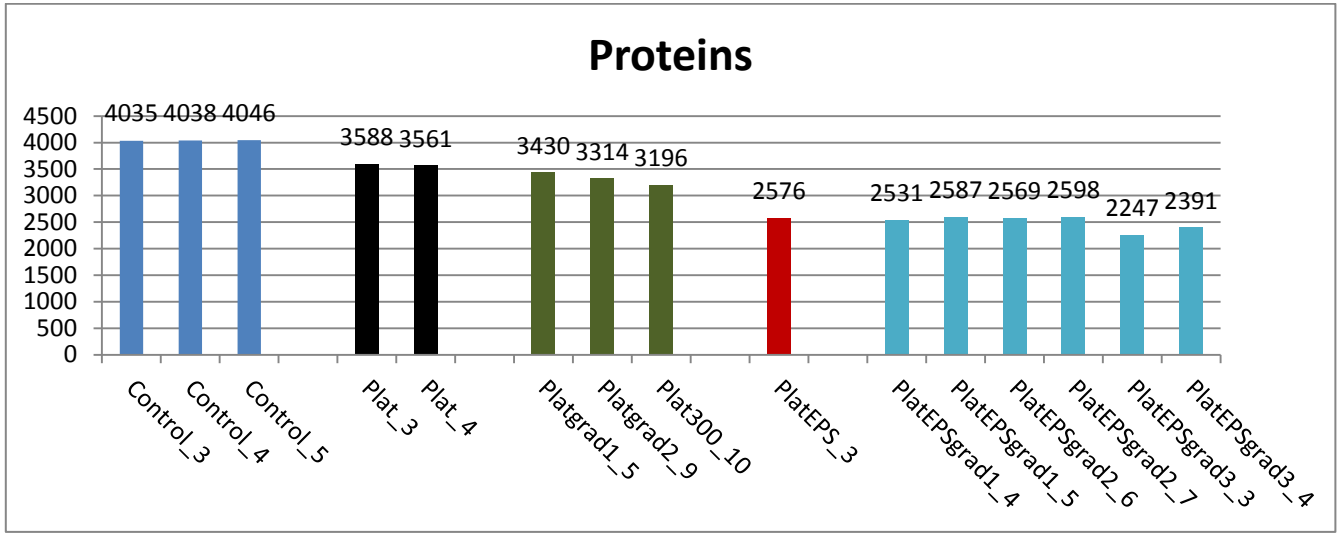


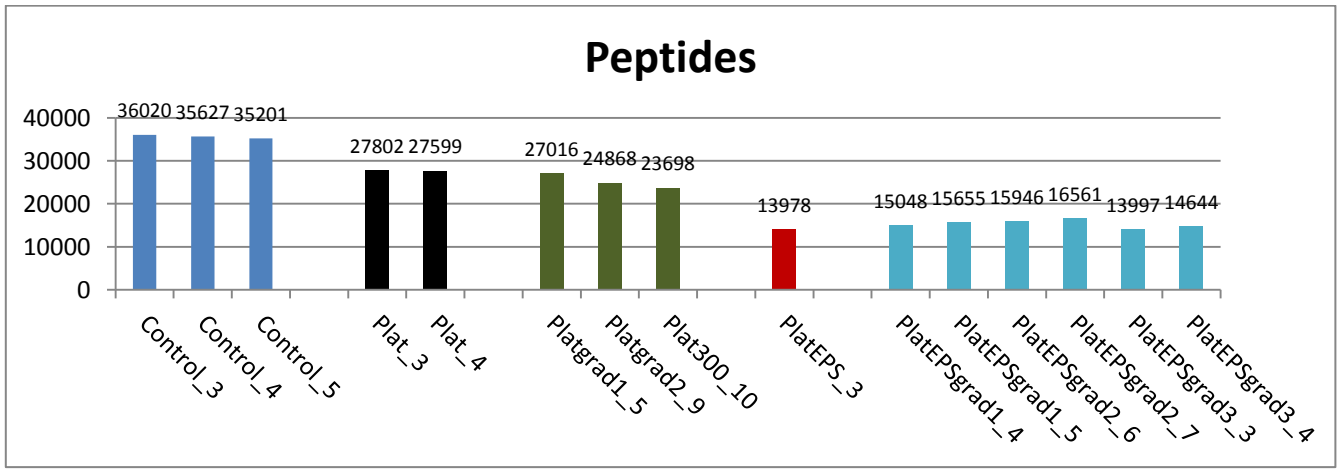
Chromatogram of 1.5 um Gold-Turbo ODS-H (GTcontrol2)



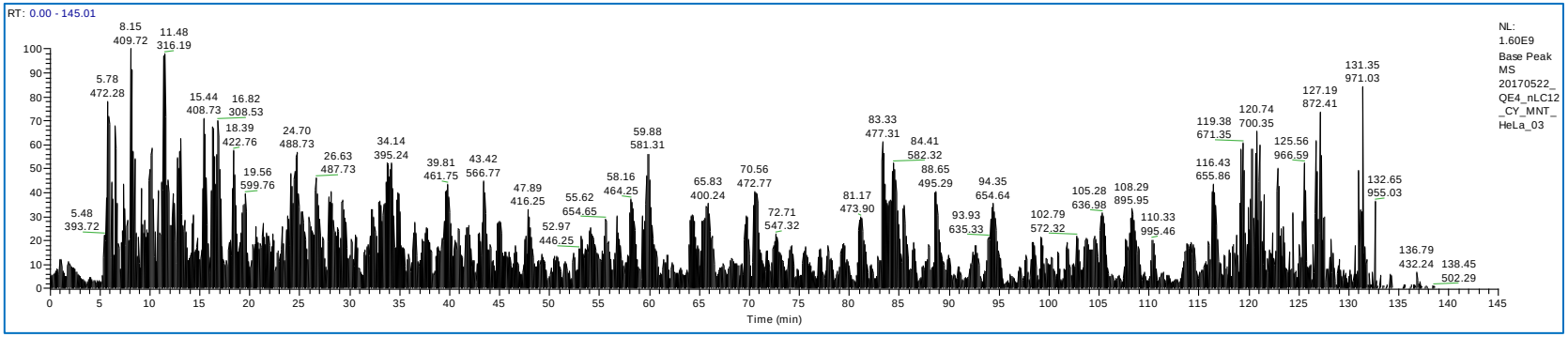
Control 1.9 um ReproSil-Pur 120 C18-AQ beads vs 1.5 um Platinum C18 beads vs 1.5 um Platinum 100 C18 EPS

Compared to the controls (blue bars), the Platinum C18 beads (black) resulted in a 10% decrease of identified proteins (top graph) and a 25% decrease in peptides (bottom graph). Alterations to the gradient (olive) did not result in any improvement. The Platinum 100 C18 EPS beads performed poorly (red), leading to considerable protein (33%) and peptide (60%) losses. Some method alterations (aqua) slightly improved the number of peptide identifications, but this generally did not change the number of overall proteins identified.

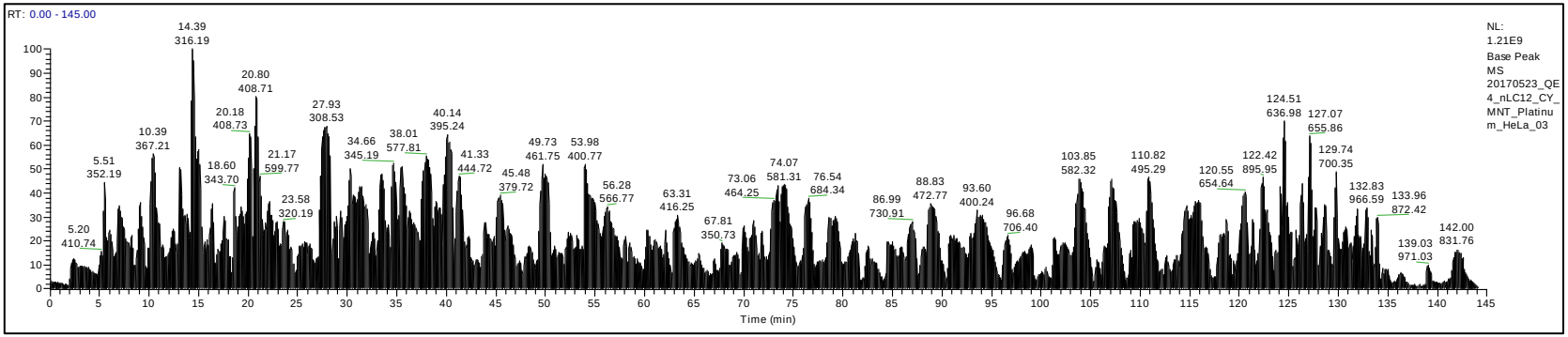




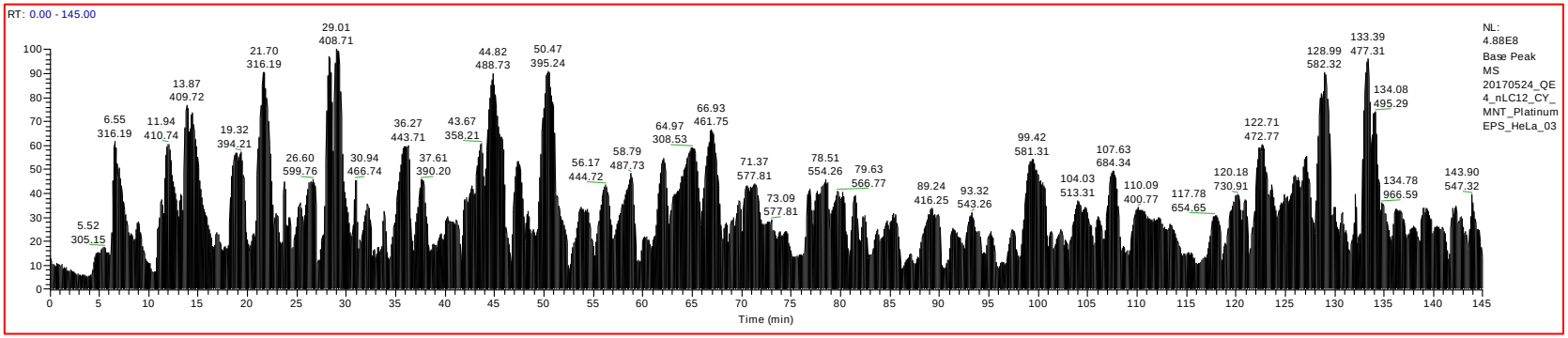
Chromatogram of 1.9 um ReproSil-Pur 120 C18-AQ (Control_3)



Chromatogram of 1.5 um Platinum C18 (Plat_3)

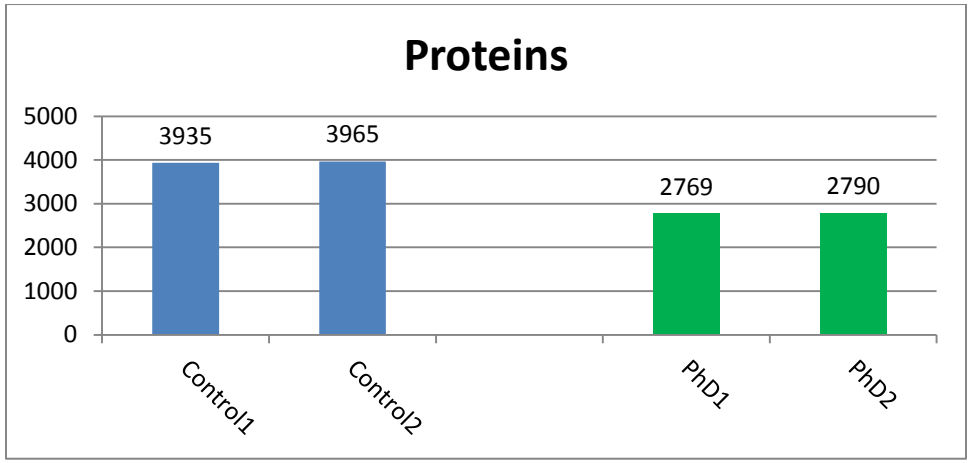


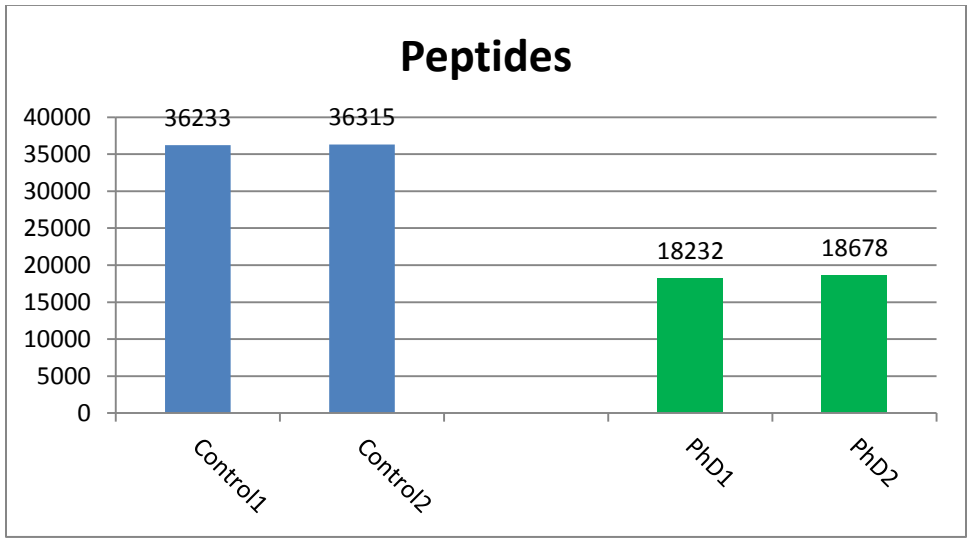
Chromatogram of 1.5 um Platinum 100 C18 (PlatEPS_3)



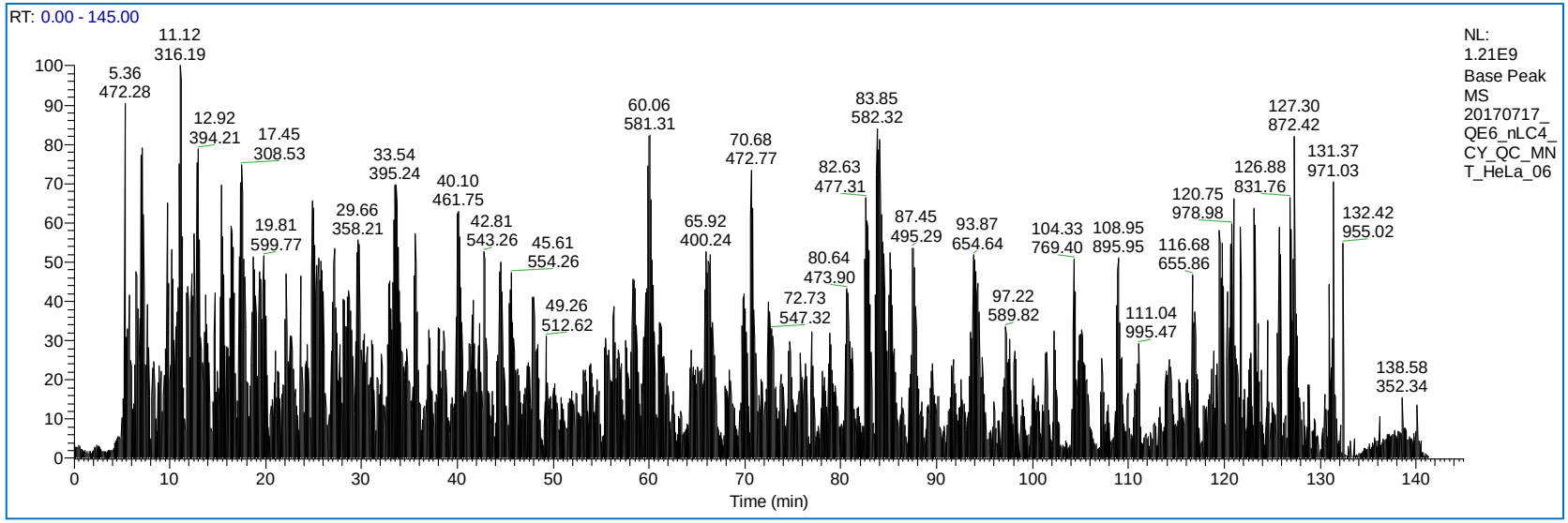
Control 1.9 um ReproSil-Pur 120 C18-AQ beads vs 1.5 um Semi Solid-Core C18 beads

Compared to the controls (blue bars), the Semi Solid-Core beads (green) resulted in a 30% decrease in identified proteins (top graph) and a 50% loss in the number of identified peptides (bottom graph).

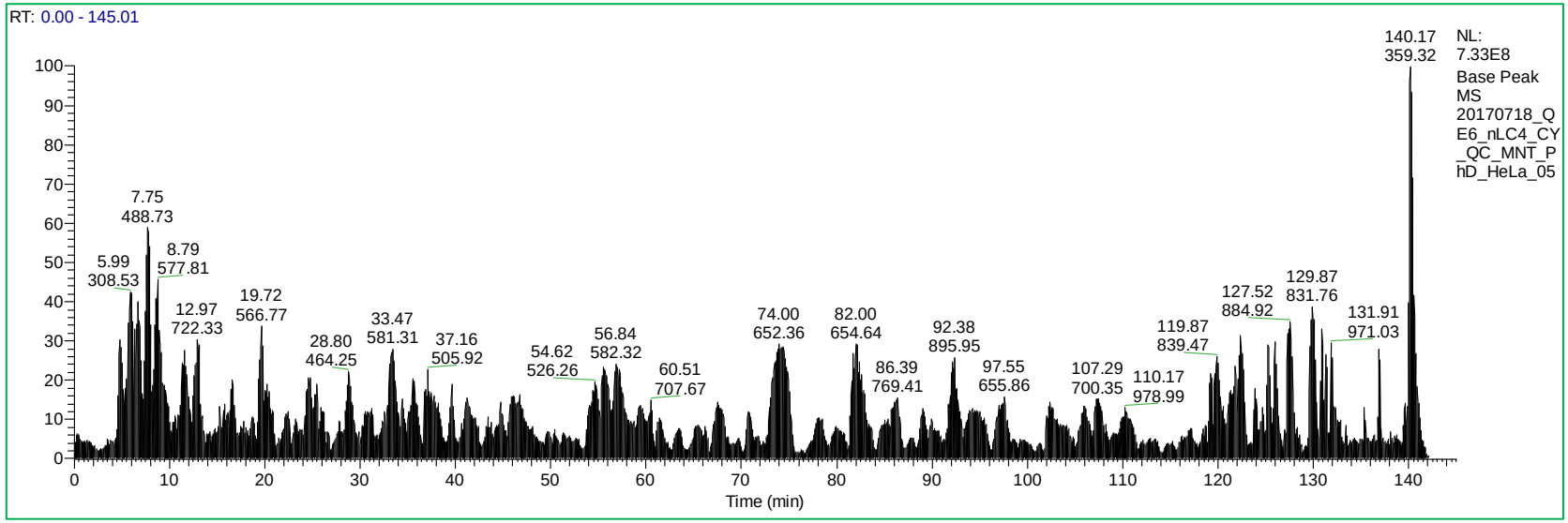




Chromatogram of 1.9 um ReproSil-Pur 120 C18-AQ (Control1)



Chromatogram of 1.5 um Semi Solid-Core C18 (PhD1)



Control 1.9 um ReproSil-Pur 120 C18-AQ beads vs 1 um Exsil Pure 120A RP18 beads

As we were only able to pack columns up to 7 cm in length with the 1 um Exsil beads, no HeLa analysis was conducted with this bead type.

Conclusions

Under the employed chromatography conditions, we were unfortunately unable to observe any improved chromatography of the HeLa peptides from the different beads. Compared to the ReproSil-Pur 120 C18-AQ beads, only the Gold-Turbo beads produced a similar chromatographical profile that resulted in comparable totals of peptide and protein identifications. Additional modifications to the gradient did not allow the performance from the Gold-Turbo beads to exceed those obtained by the controls. It is therefore evident that the chemistry relating to the production of the ReproSil-Pur beads produces a high level of chromatographical performance that is difficult to surpass. We also acknowledge that the different beads may have been developed for vastly different chromatographic conditions and purposes that are quite distinct from our analysis of complex peptide samples by LC-MS.

If you have any questions, please do not hesitate to contact us.

Yours sincerely,

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