High percentage protein identification by nanoLC-MS/MS after efficient peptide desalting on C18 Stage Tips with a broad capacity range

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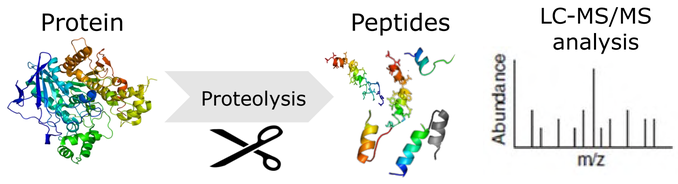
Context

Bottom-up workflow for proteomics



Salts in digestion buffers

- Generate **ion-suppression**
- **Limit peptide detection**
- **Impact proteins identification**

Challenge

Peptide desalting and purification before LC-MS/MS analysis

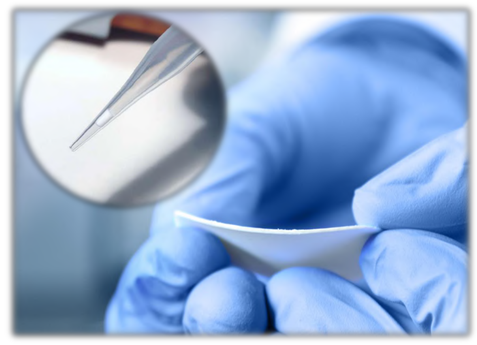
- ✓ **Salts** must be **efficiently removed**
- ✓ **Hydrophilic and hydrophobic peptides** must be **retained** to preserve all **useful information** about the sample

Solution

AttractSPE®Disks Tips C18

Among a large diversity of C_{18} powders, a **C_{18} with a wide spectrum of interactions** was selected to **interact with the broadest range of peptides**. Small sorbent particles embedded in a monolithic disk were used to combine **high capacity** and **small dead volume.**

These C_{18} disks were tested into **home-made SPE tips** (called **Stage Tips**) to evaluate performances in desalting HeLa digest before peptides analysis by nanoLC-MS/MS.



Principle of AttractSPE® Disks

AttractSPE®Disks are soft, thin, uniform and mechanically stable membranes for the extraction, purification and concentration of analytes in proteomics studies.

AttractSPE®Disks membranes are SPE sorbents able to efficiently capture analytes and to easily release them for analysis, if required.

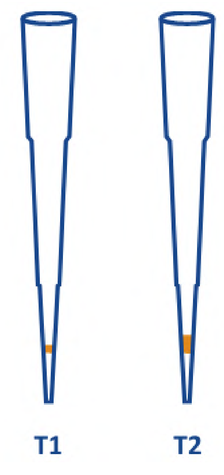
Sorbent particles are tightly packed with no void space	**Minimized diffusion distance between particles**	**Thin membrane, small bed volume**
↓	↓	↓
No need of frits Reduced dead volume	**Small elution volumes**	**High sample recovery with excellent repeatability**

Evaluation of C18 sorbent

Several new **C18 Stage Tips**, made with different thicknesses of **AttractSPE®Disks C18** and therefore different capacities (T1 or T2), were evaluated for peptide desalting. These **AttractSPE®Disks Tips C18** are particularly adapted for **centrifugation** or positive pressure assays.

T1: A layer of C18 sorbent with a thickness equivalent to **ONE** layer of SPE disks (around 0.6mm)

T2: A layer of C18 sorbent with a thickness equivalent to **TWO** layers of SPE disks (around 1.2mm)



Protocol of use for AttractSPE®Disks Tips C18

Processing step*	Operation	Centrifuge – time and speed
1 - Conditioning	100µl 70% ACN 0.1% FA	1min – 2,000 RPM (800 x *g*)
2 – Equilibration	100µl 0.1% FA	1min – 2,000 RPM (800 x *g*)
3 – Loading of sample	1.1ng, 11ng, 110ng, 1µg, 5µg or 10µg HeLa Digest in 100µl 0.1% FA	1min – 2,000 RPM (800 x *g*)
4 - Washing	100µl 0.1% FA	1min – 2,000 RPM (800 x *g*)
5 - Elution	100µl 40% ACN 0.1% FA	1min – 2,000 RPM (800 x *g*)
6 – Evaporation	Speed Vacuum dried	
7 - Reconstitution	Samples resuspended in 5.5µl (with IRT) and 5µl (1,10 or 100ng) injected in LC-MS/MS	

*Same protocol used for all the AttractSPE®Disks Tips, regardless of their capacity

LC-MS/MS analysis of peptides

NanoLC conditions

Trap column: nanoViper Acclaim PepMap™ C18 (2cm x 75µm)

Column: Acclaim PepMap™ RSLC C18 (50cm x 75µm, 2µm)

Temperature: 40°C

Gradient: 3% to 29% buffer B over 91min at a flow rate of 300 nL/min

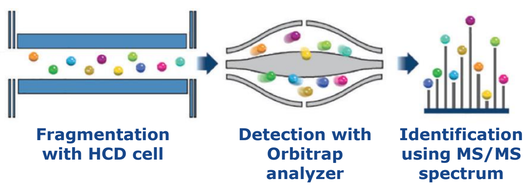
Buffer A: 2/98 ACN/H_2O in 0.1% FA

Buffer B: ACN in 0.1% FA

MS/MS conditions (Orbitrap Exploris 480)

MS full scans in ranges ***m/z* 375-1500**

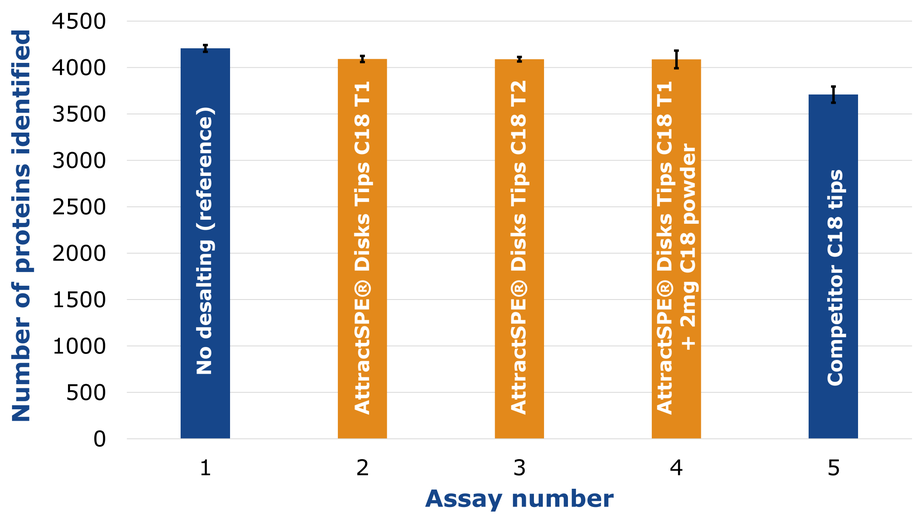
Top 20 most intense ions isolated and fragmented by HCD



Protein identification with AttractSPE®Disks Tips C_{18}

Three **AttractSPE® Disks Tips C18** with different capacities were tested for the desalting of 100ng of HeLa digest and were compared to a major competitor product (n=5 for each assay)

Assay number	Description
1	No purification (reference assay)
2	Desalting on AttractSPE®Disks Tips C18 200µL - **T1**
3	Desalting on AttractSPE®Disks Tips C18 200µL - **T2**
4	Desalting on AttractSPE®Disks Tips C18 200µL - **T1 + 2mg powder** AttractSPE® C18
5	Desalting on competitor C18 tips (100µL bed)



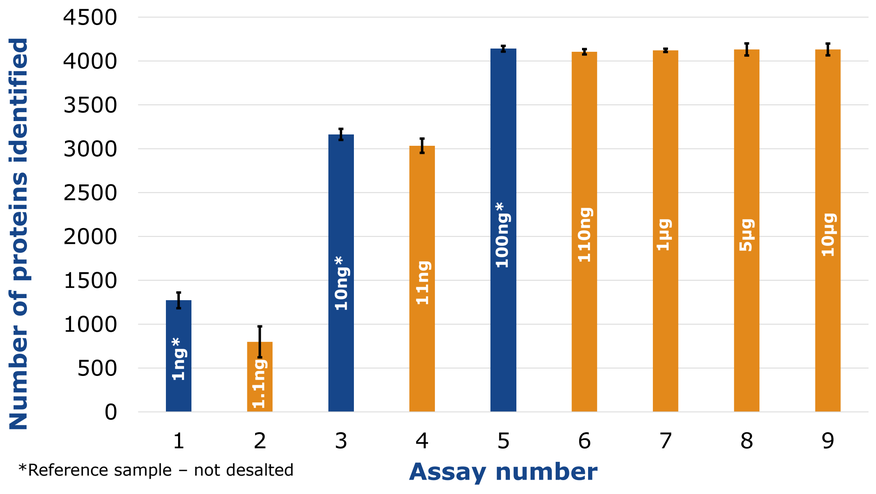
Results

- ✓ **Identification of 97% of proteins** after desalting of HeLa digest on **AttractSPE®Disks Tips C18** (better results than competitor tips)
- ✓ Similar results obtained for the three **AttractSPE®Disks Tips C18** : **all retained peptides are released from the tips**, regardless of the sorbent amount
- ✓ Good **retention of peptides on the whole polarity range**, from the most hydrophobic to the most polar
- ✓ **Very low variability** observed for intra- and inter- tips assays: reliability of **AttractSPE®Disks Tips C18**

Working range of AttractSPE®Disks Tips C_{18} T1

1ng to 10µg of HeLa digest were loaded on the **AttractSPE®Disks Tips C18 T1** to determine their working range (n=3 for each assay)

Assay number	Amount of HeLa digest desalted	Amount analyzed by LC-MS/MS
1	-	1ng – not desalted as reference
2	1.1ng	1ng
3	-	10ng – not desalted as reference
4	11ng	10ng
5	-	100ng – not desalted as reference
6	110ng	100ng
7	1µg	100ng
8	5µg	100ng
9	10µg	100ng



*Reference sample – not desalted

Results

- ✓ **Identification of 95% of proteins** after desalting of HeLa digest amounts ranging from 10ng to 10µg on **AttractSPE®Disks Tips C18 T1**, with **RSD < 3%**
- ✓ No loss of performance for 10µg digest: **maximal capacity** of **AttractSPE®Disks Tips C18 T1 not reached**
- ✓ For 1ng of digest, the number of identified proteins remains very high despite the low amount of material: **AttractSPE®Disks Tips C18 T1 could be used for single cell-like analysis**

Conclusion

AttractSPE®Disks C18 sorbent can be used for **efficient peptide desalting** and is available as **Stage Tips**, **Spin columns**, **96 well plates** and **SPE cartridges**

☑ **No loss of peptide** ☑ **High capacity** ☑ **Excellent repeatability** ☑ **Simplicity of use** ☑ Broad range of use: from **single cell to high peptide amounts**

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