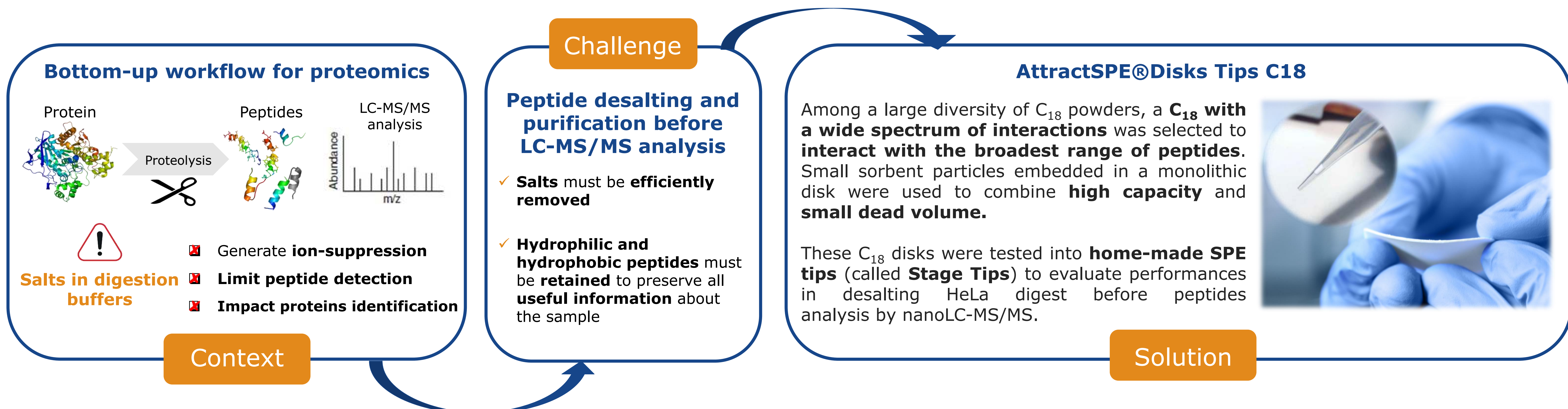


# High percentage protein identification by nanoLC-MS/MS after efficient peptide desalting on C18 Stage Tips with a broad capacity range

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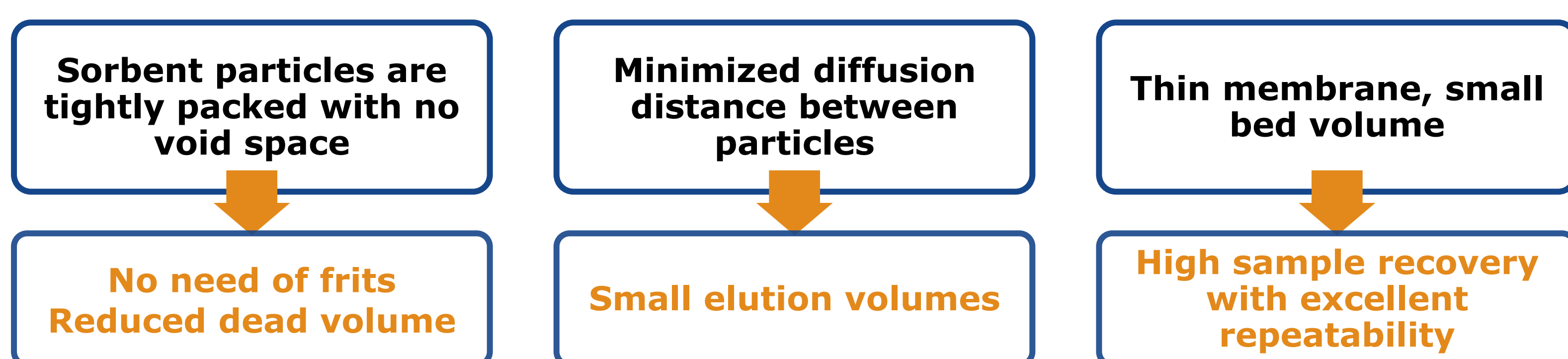
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## Principle of AttractSPE® Disks

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.



## 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	

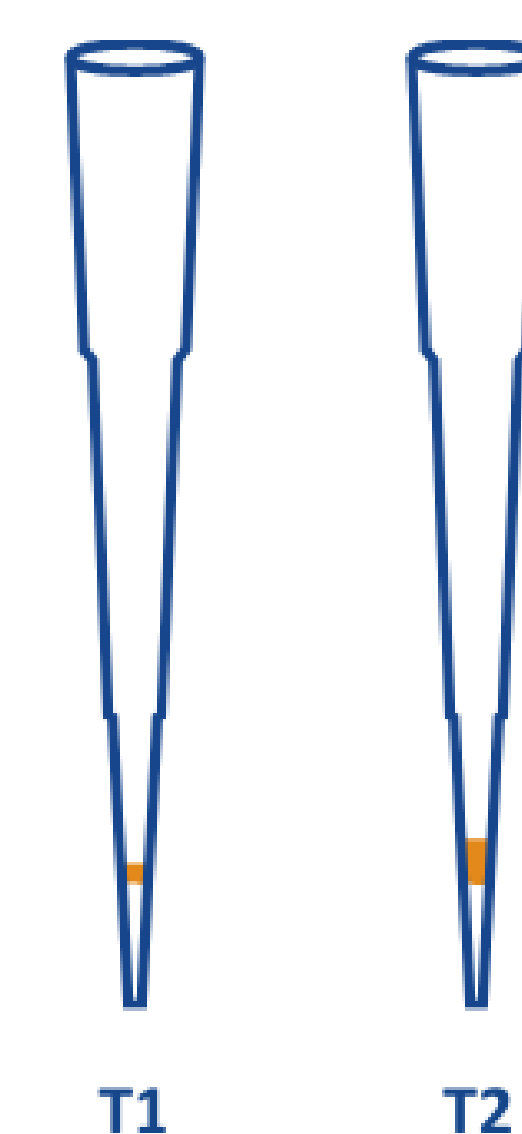
\*Same protocol used for all the AttractSPE® Disks Tips, regardless of their capacity

## Evaluation of C18 sorbent

Several new C<sub>18</sub> Stage Tips, made with different thicknesses of AttractSPE® Disks C<sub>18</sub> and therefore different capacities (T1 or T2), were evaluated for peptide desalting. These AttractSPE® Disks Tips C<sub>18</sub> are particularly adapted for centrifugation or positive pressure assays.

**T1:** A layer of C<sub>18</sub> sorbent with a thickness equivalent to ONE layer of SPE disks (around 0.6mm)

**T2:** A layer of C<sub>18</sub> sorbent with a thickness equivalent to TWO layers of SPE disks (around 1.2mm)



## 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

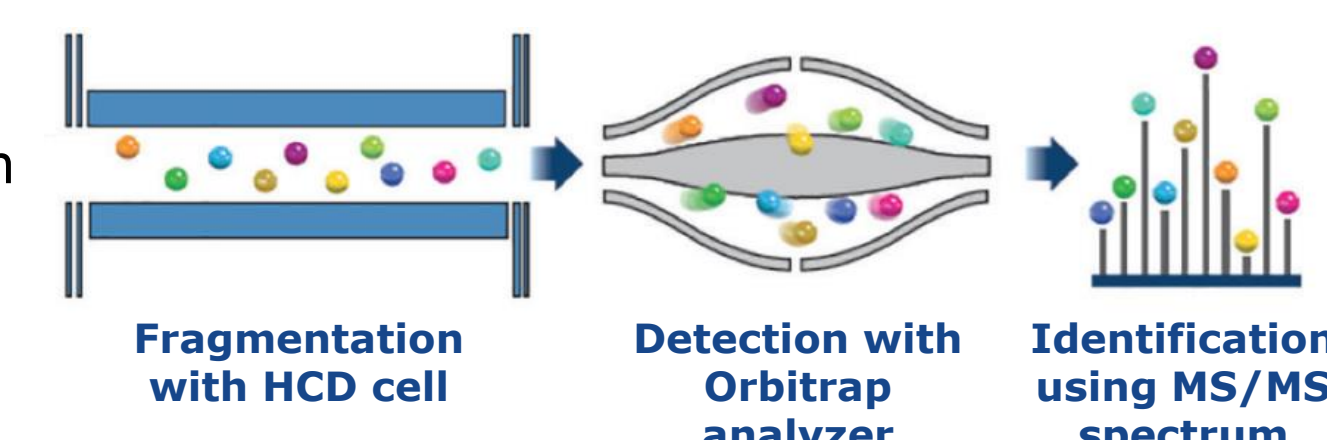
Buffer A: 2/98 ACN/H<sub>2</sub>O in 0.1% FA

Buffer B: ACN in 0.1% FA

### MS/MS conditions (Orbitrap Exploris 480)

MS full scans in ranges m/z 375-1500

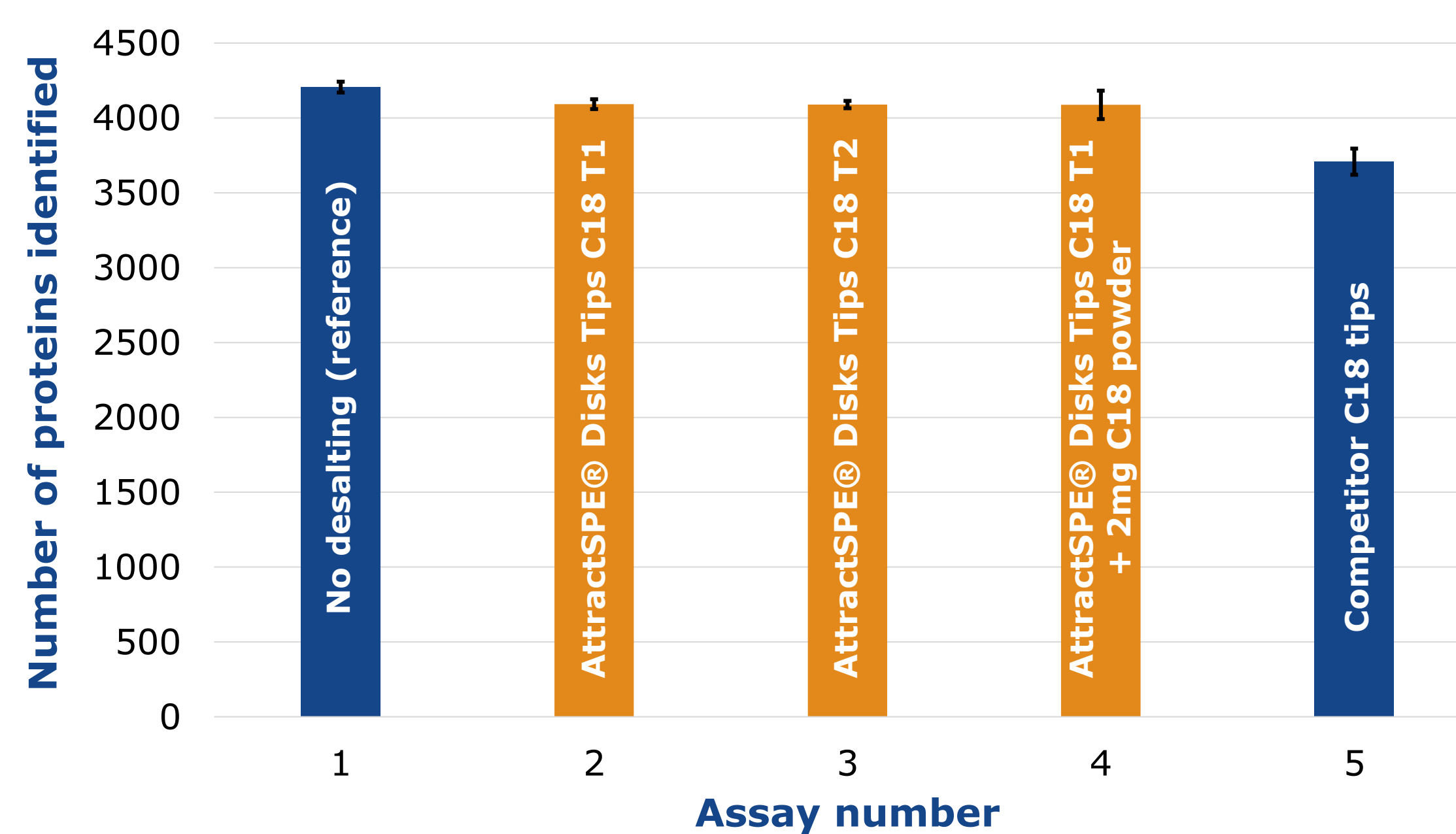
Top 20 most intense ions isolated and fragmented by HCD



## Protein identification with AttractSPE® Disks Tips C18

Three AttractSPE® Disks Tips C<sub>18</sub> 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 C <sub>18</sub> 200µL - T1
3	Desalting on AttractSPE® Disks Tips C <sub>18</sub> 200µL - T2
4	Desalting on AttractSPE® Disks Tips C <sub>18</sub> 200µL - T1 + 2mg powder AttractSPE® C <sub>18</sub>
5	Desalting on competitor C <sub>18</sub> tips (100µL bed)



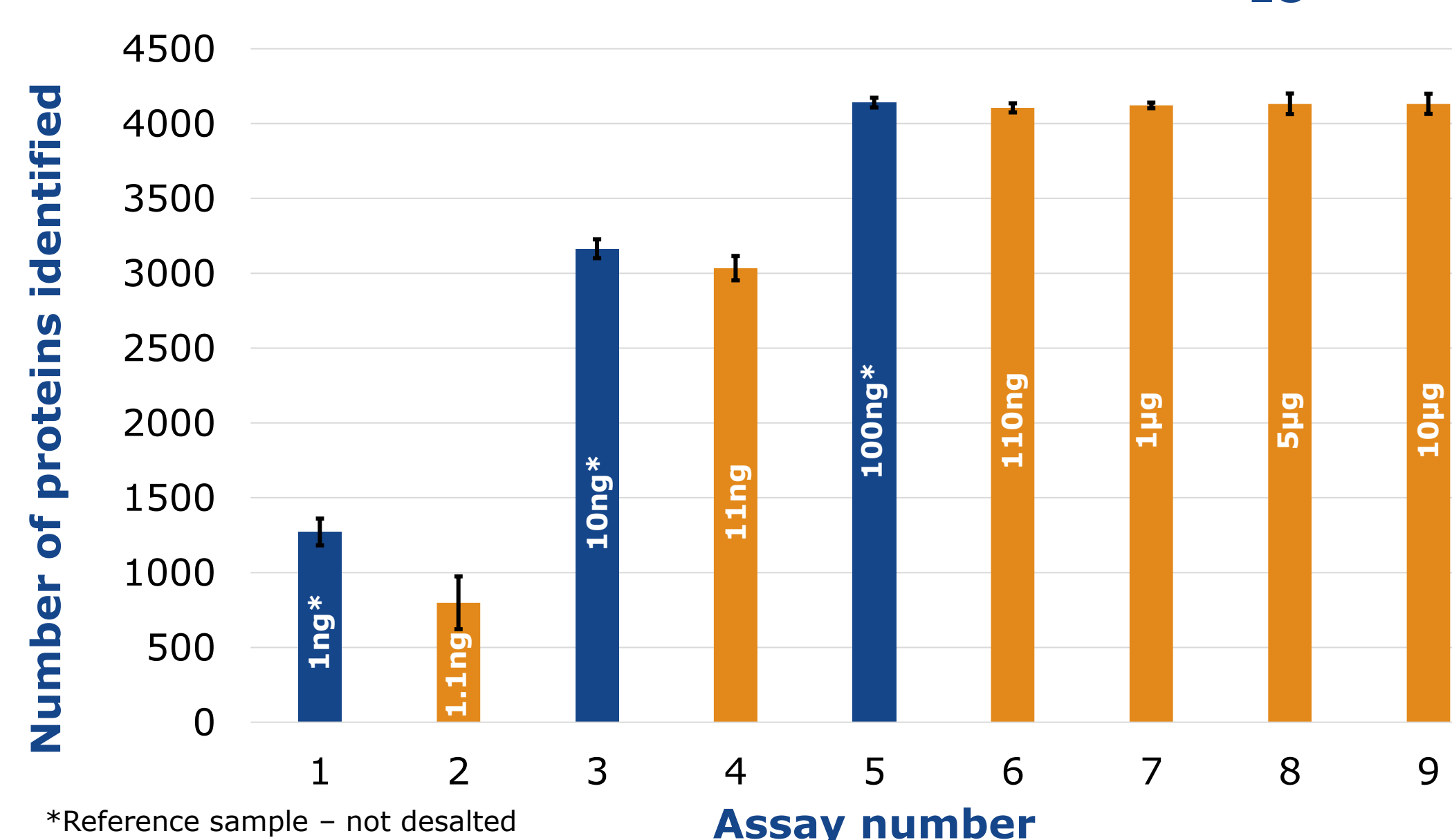
### Results

- Identification of 97% of proteins after desalting of HeLa digest on AttractSPE® Disks Tips C<sub>18</sub> (better results than competitor tips)
- Similar results obtained for the three AttractSPE® Disks Tips C<sub>18</sub>: 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 C<sub>18</sub>

## Working range of AttractSPE® Disks Tips C18 T1

1ng to 10µg of HeLa digest were loaded on the AttractSPE® Disks Tips C<sub>18</sub> 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 C<sub>18</sub> T1, with RSD < 3%
- No loss of performance for 10µg digest: maximal capacity of AttractSPE® Disks Tips C<sub>18</sub> T1 not reached
- For 1ng of digest, the number of identified proteins remains very high despite the low amount of material: AttractSPE® Disks Tips C<sub>18</sub> T1 could be used for single cell-like analysis

## Conclusion

AttractSPE® Disks C<sub>18</sub> 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