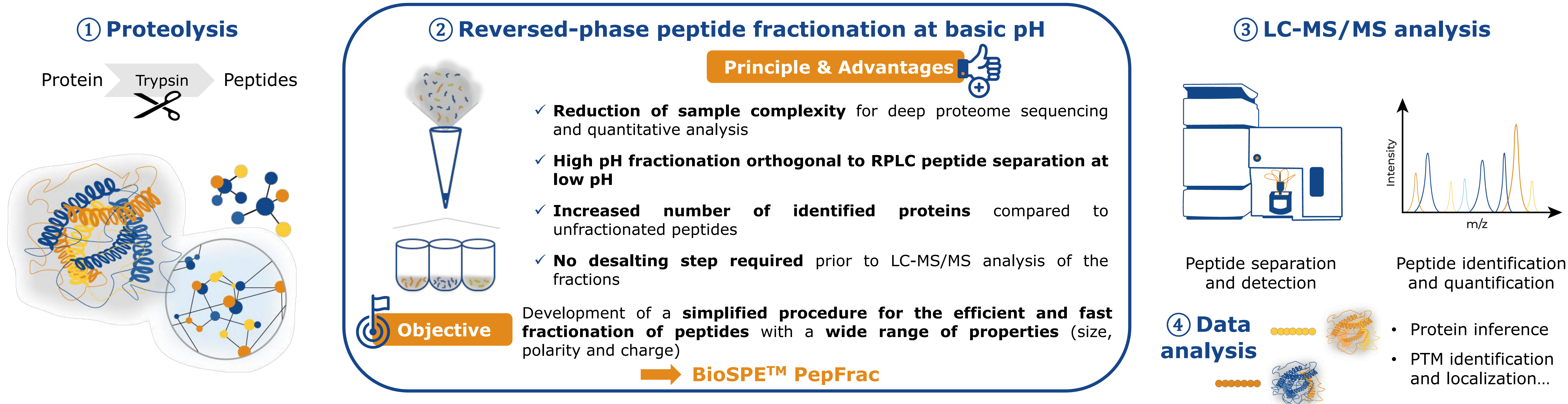


A new SPE Tips method based on an innovative sorbent for fast and efficient peptide fractionation in proteomic studies

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Context: bottom-up proteomics workflow

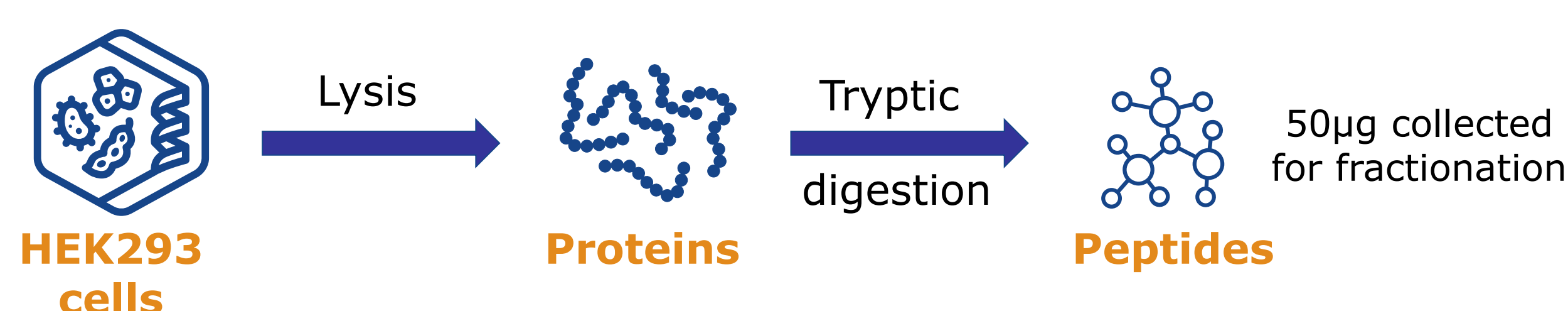


Evaluation of BioSPE™ PepFrac for peptide fractionation at basic pH

① What is BioSPE™ PepFrac?

BioSPE™ PepFrac is a new **reversed-phase sorbent** based on Affinisep disks technology, specifically developed for the fractionation of peptides in proteomics studies. In this study, **BioSPE™ PepFrac 200µL Tips** were evaluated for the **fractionation of peptides resulting from the enzymatic digestion of proteins contained in HEK293 cell lysate**. Results were compared with a competitor fractionation column.

② HEK cell lysis and protein digestion

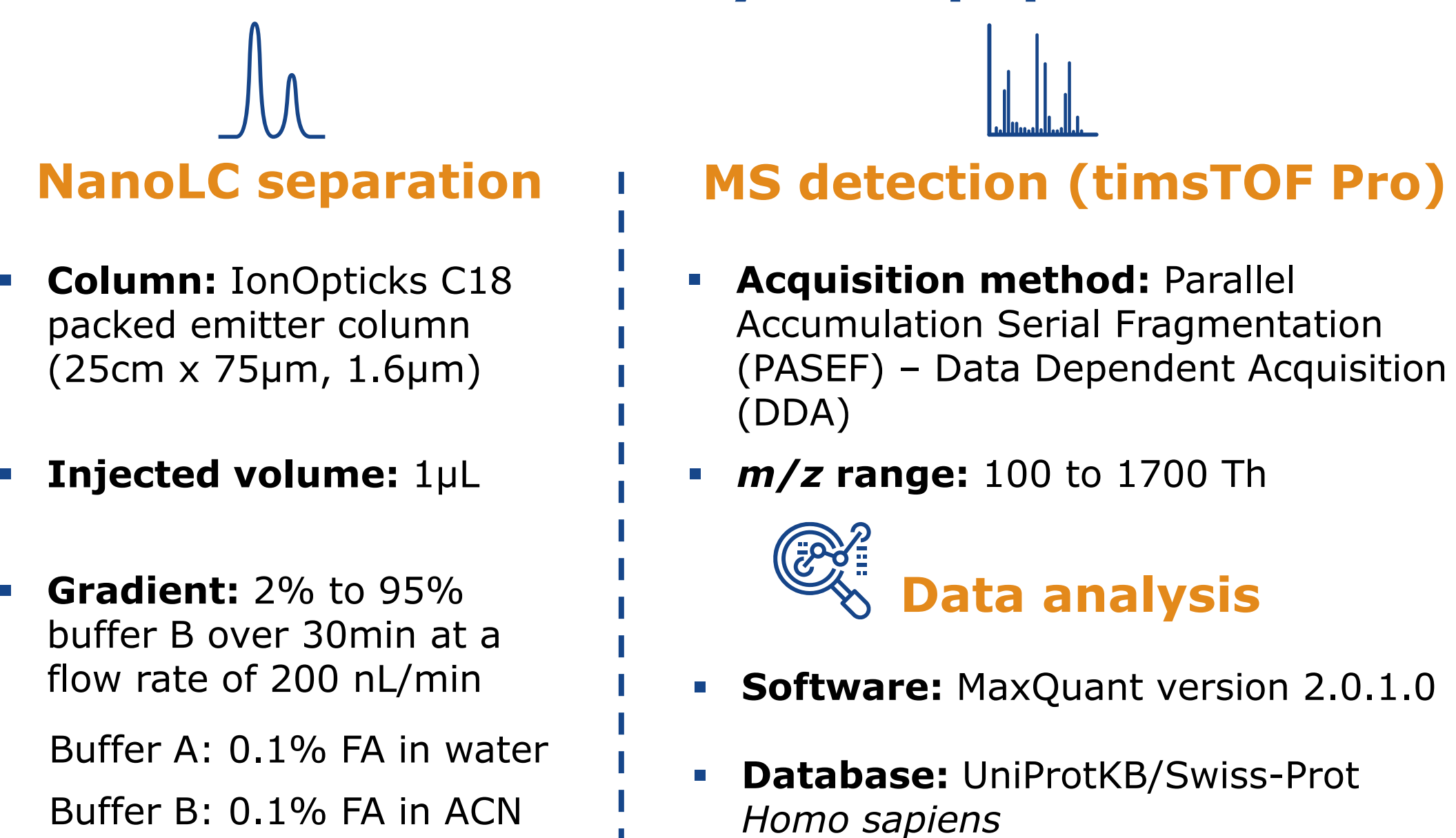


③ Peptide fractionation protocol at basic pH & LC-MS/MS analysis conditions

Fractionation protocol with ACN gradient

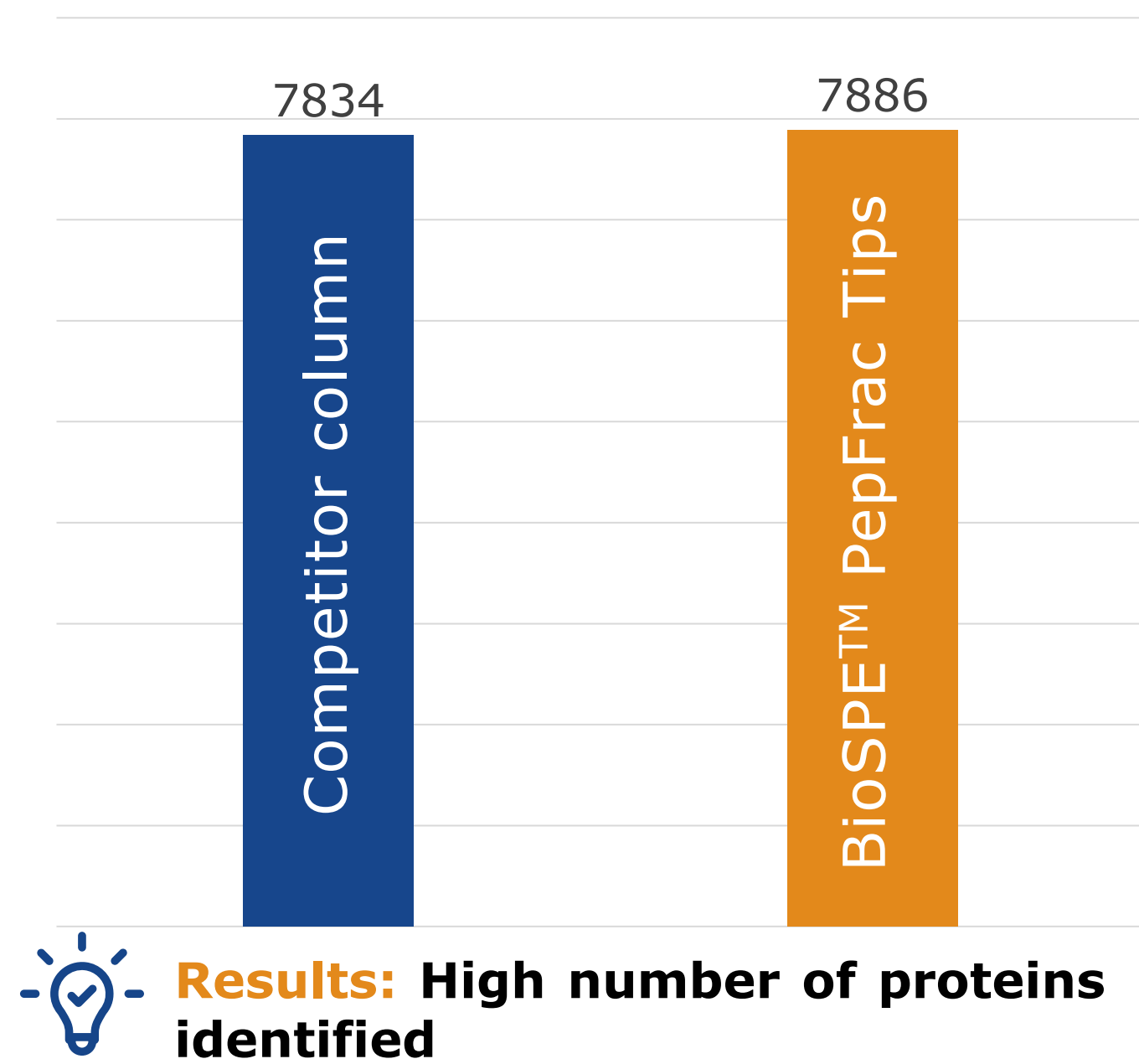
| Processing step | BioSPE™ PepFrac Tips | Competitor column |
|-------------------|--|--|
| Conditioning | 2x 150µL ACN - 1,500g - 2min | 2x 300µL ACN - 5,000g - 2min |
| Equilibration | 2x 150µL 0.1% TFA - 1,500g - 2min | 2x 300µL 0.1% TFA - 5,000g - 2min |
| Loading of sample | 150µL - 1,500g - 2min | 300µL - 3,000g - 2min |
| Washing | 150µL H ₂ O - 1,500g - 2min | 300µL H ₂ O - 3,000g - 2min |
| Fraction 1 | 150µL ACN/TEA 0.1% (2/98) - 1,500g - 2min | 300µL ACN/TEA 0.1% (5/95) - 3,000g - 2min |
| Fraction 2 | 150µL ACN/TEA 0.1% (4/96) - 1,500g - 2min | 300µL ACN/TEA 0.1% (7.5/92.5) - 3,000g - 2min |
| Fraction 3 | 150µL ACN/TEA 0.1% (6/94) - 1,500g - 2min | 300µL ACN/TEA 0.1% (10/90) - 3,000g - 2min |
| Fraction 4 | 150µL ACN/TEA 0.1% (8/92) - 1,500g - 2min | 300µL ACN/TEA 0.1% (12.5/87.5) - 3,000g - 2min |
| Fraction 5 | 150µL ACN/TEA 0.1% (10/90) - 1,500g - 2min | 300µL ACN/TEA 0.1% (15/85) - 3,000g - 2min |
| Fraction 6 | 150µL ACN/TEA 0.1% (12/88) - 1,500g - 2min | 300µL ACN/TEA 0.1% (17.5/82.5) - 3,000g - 2min |
| Fraction 7 | 150µL ACN/TEA 0.1% (15/85) - 1,500g - 2min | 300µL ACN/TEA 0.1% (20/80) - 3,000g - 2min |
| Fraction 8 | 150µL ACN/TEA 0.1% (50/50) - 1,500g - 2min | 300µL ACN/TEA 0.1% (50/50) - 3,000g - 2min |
| Evaporation | SpeedVac (2h) | SpeedVac (3h30) |
| Resuspension | 13µL 0.1%FA | 13µL 0.1%FA |

LC-MS/MS analysis of peptides

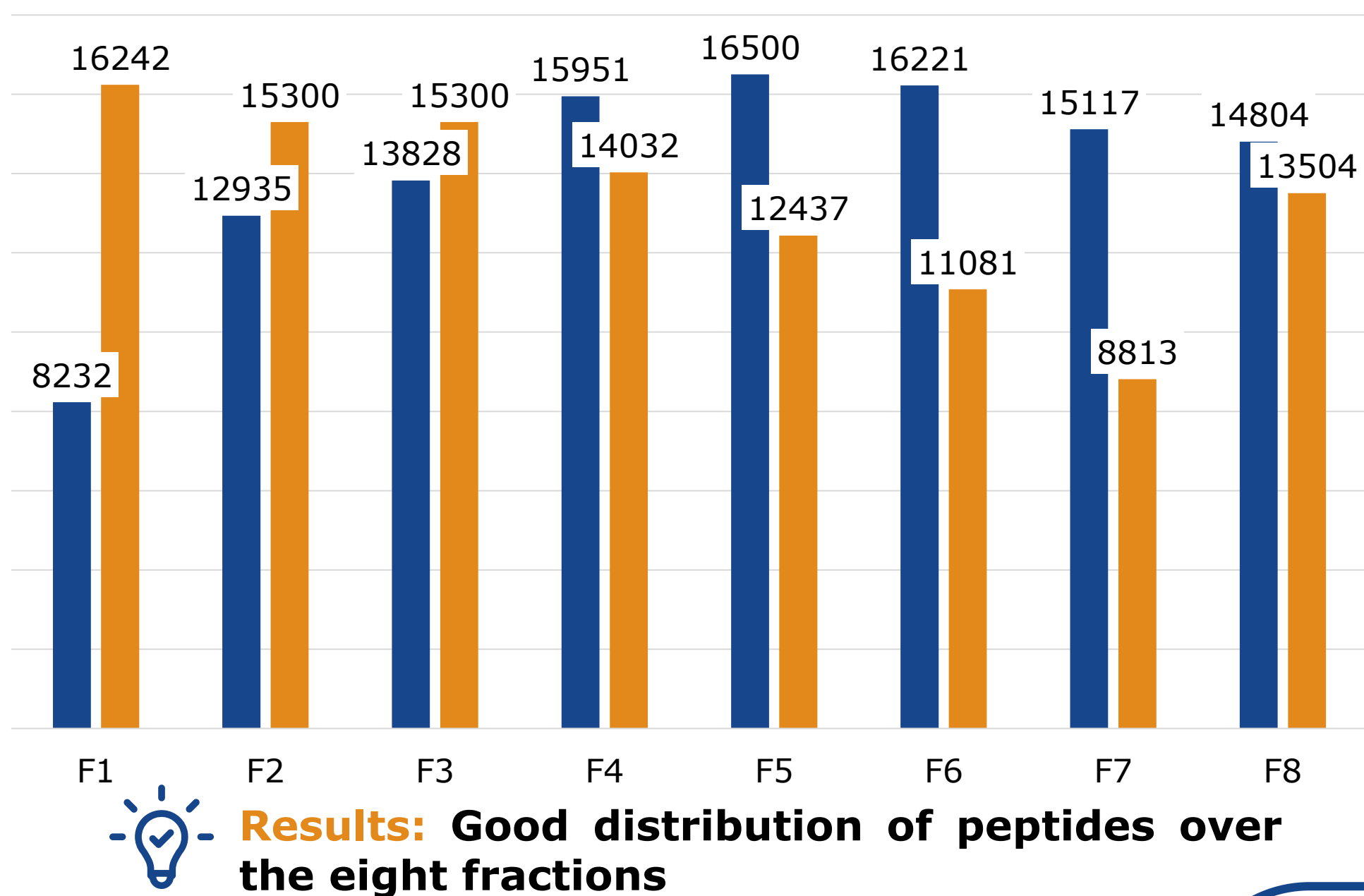


④ Results of peptide fractionation on BioSPE™ PepFrac Tips & comparison with competitor fractionation column

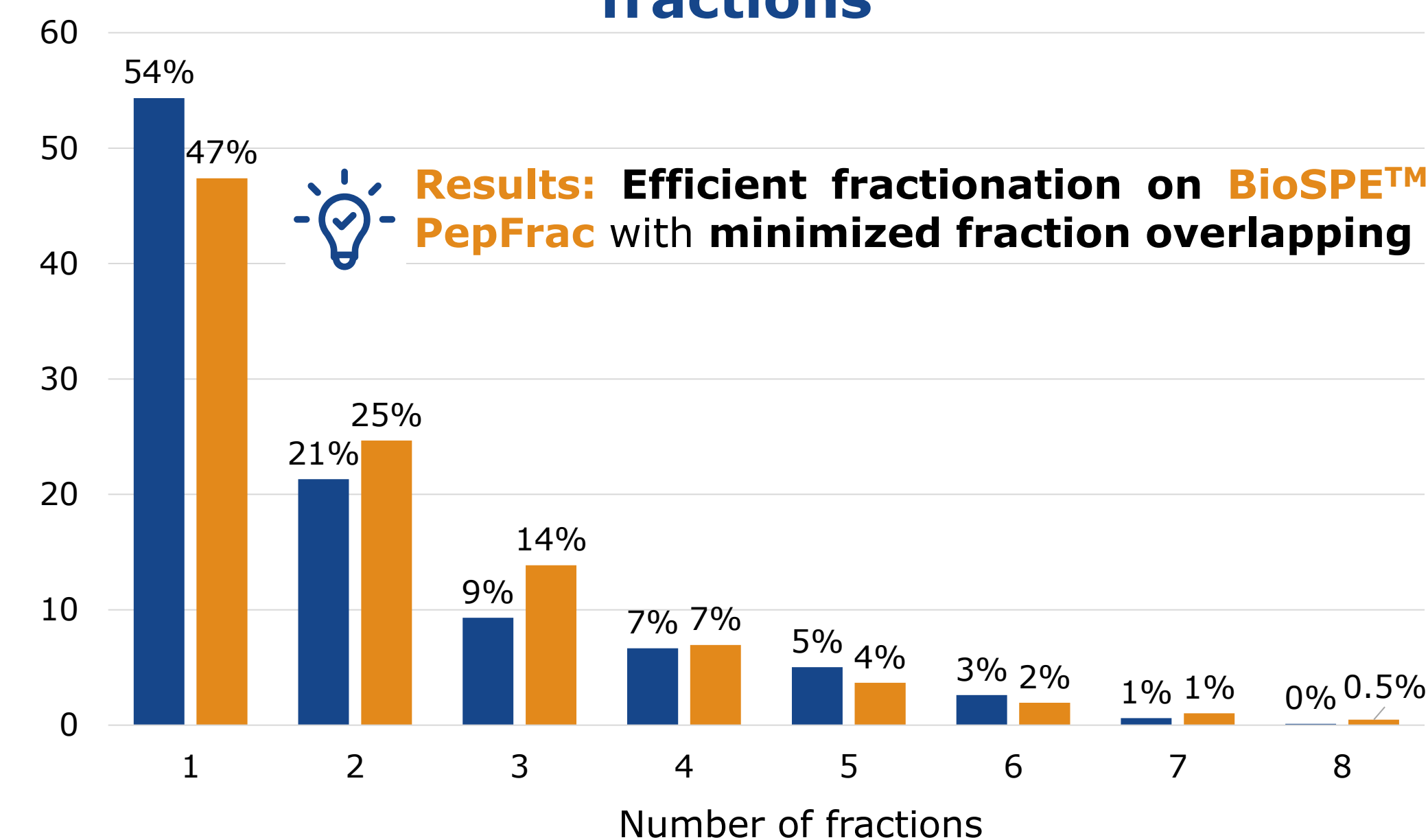
Total number of proteins identified



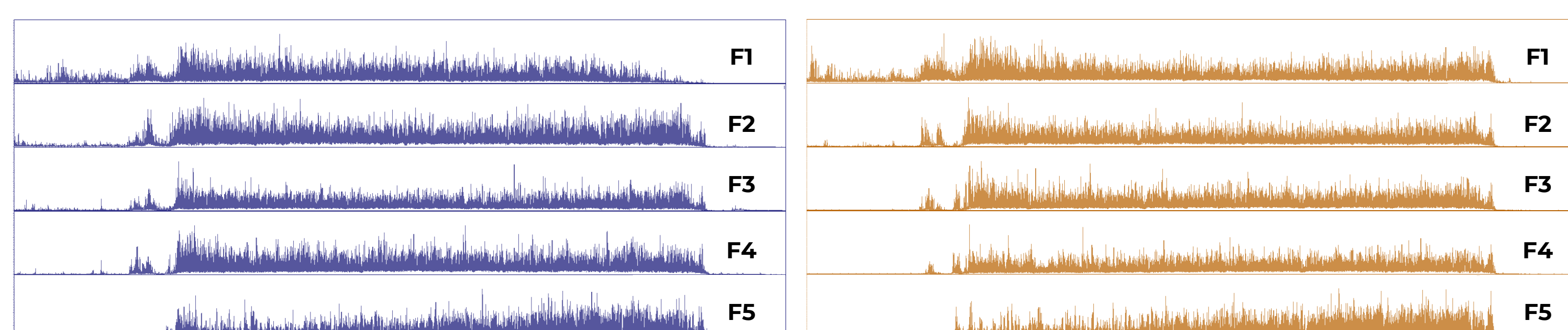
Peptide distribution in each fraction



Percentage of peptides eluting in several fractions



Spectral density over HPLC gradient



Results: Good repartition of peptides over analytical run

Advantages of BioSPE™ PepFrac

- ✓ **No storage constraints for BioSPE™ PepFrac (dry at room temperature for several years)** contrary to competitor column (4°C, in storage buffer)
- ✓ **Time required for evaporation of each fraction almost halved with BioSPE™ PepFrac Tips**
- ✓ **Fractionation of 10 to 50µg of peptides on BioSPE™ PepFrac 200µL Tips**
- ✓ **Flexibility of format and capacity: BioSPE™ PepFrac available as spin columns for higher peptide amounts or 96 wellplates for high throughput experiments**

Conclusion

BioSPE™ PepFrac appears as a promising alternative to the competitor fractionation column, especially for **complex samples such as plasma** or the **generation of spectral libraries**, since it leads to an **increase of more than 27% in the number of proteins identified**, compared to unfractionated samples.