

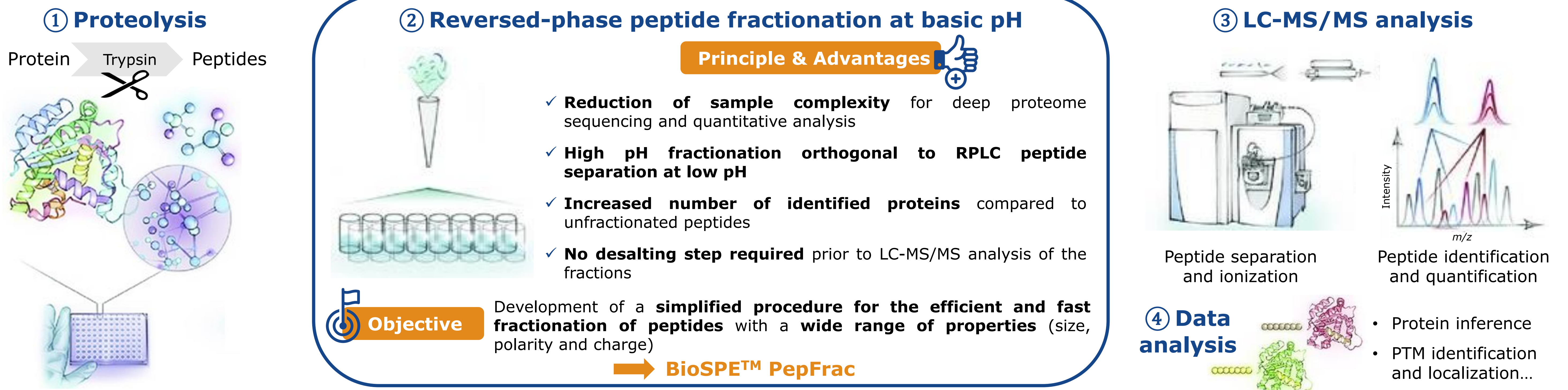
A new reversed-phase resin for fast and efficient peptide fractionation at basic pH in proteomic studies

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Context: bottom-up proteomics workflow

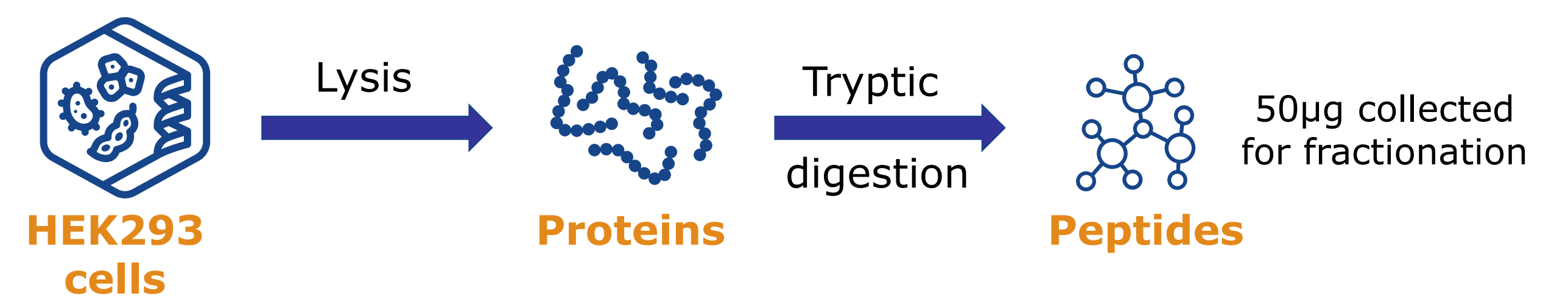


Evaluation of BioSPE™ PepFrac for peptide fractionation at basic pH

① What is BioSPE™ PepFrac?

BioSPE™ PepFrac is a new **reversed-phase resin** 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

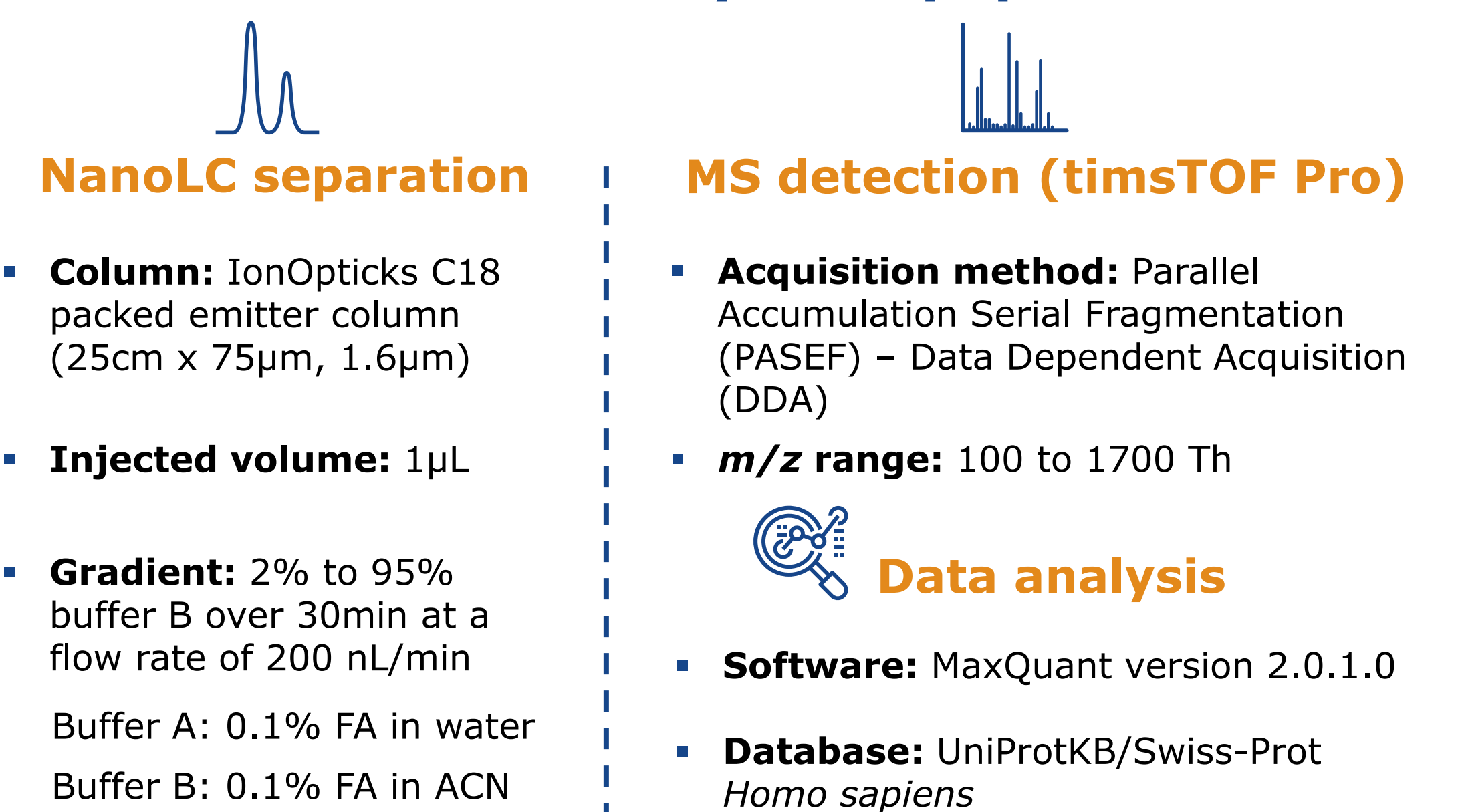


③ Peptide fractionation protocol at basic pH & LC-MS/MS analysis conditions

Fractionation protocol with ACN gradient

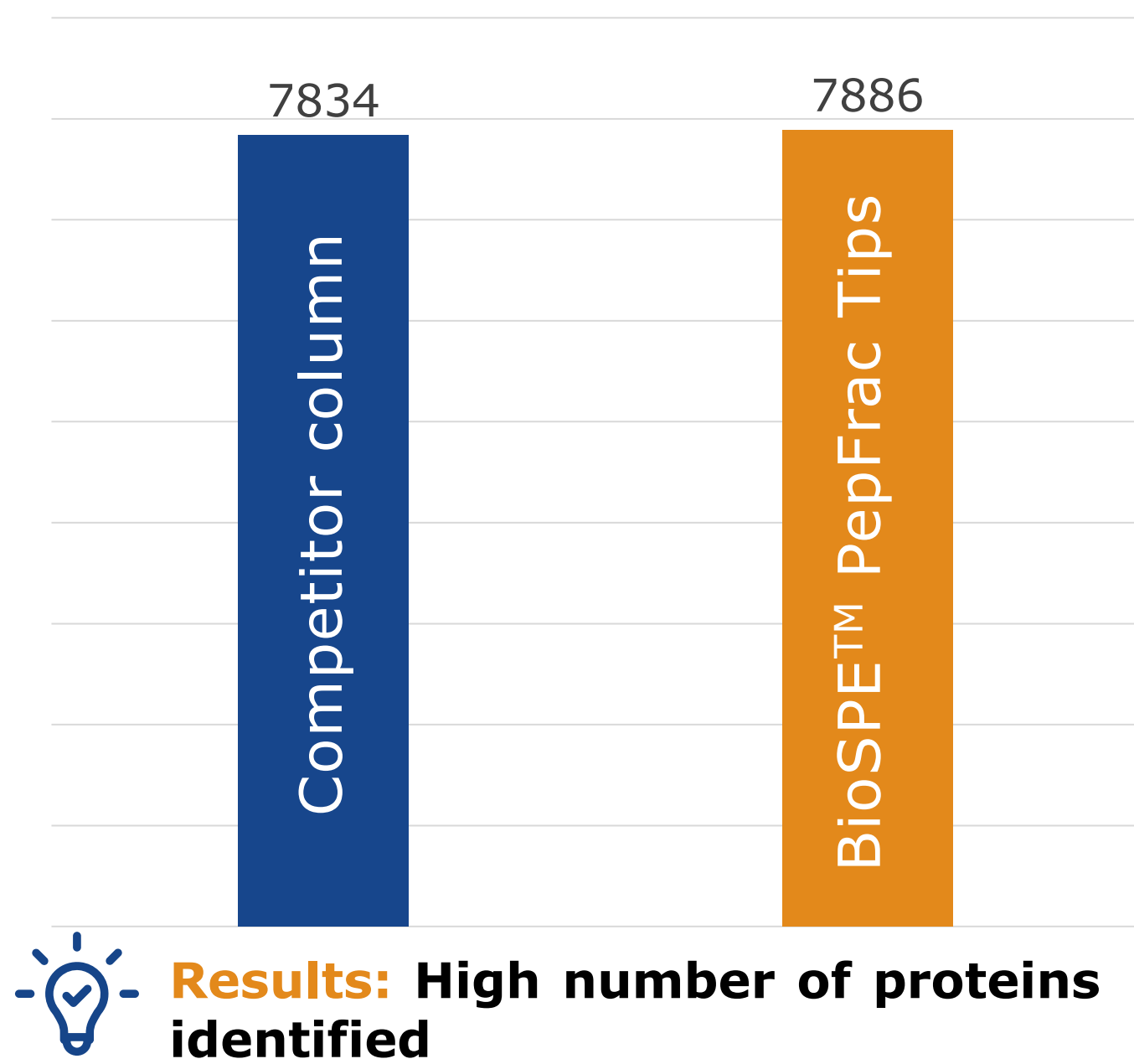
Processing step	BioSPE™ PepFrac Tips	Competitor column
Conditioning	2x 150µL ACN - 1,500g - 2min	2x 300µL ACN - 5,000g - 2min
Equilibration	2x 150µL 0.1% TFA - 1,500g - 2min	2x 300µL 0.1% TFA - 5,000g - 2min
Loading of sample	150µL - 1,500g - 2min	300µL - 3,000g - 2min
Washing	150µL H ₂ O - 1,500g - 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

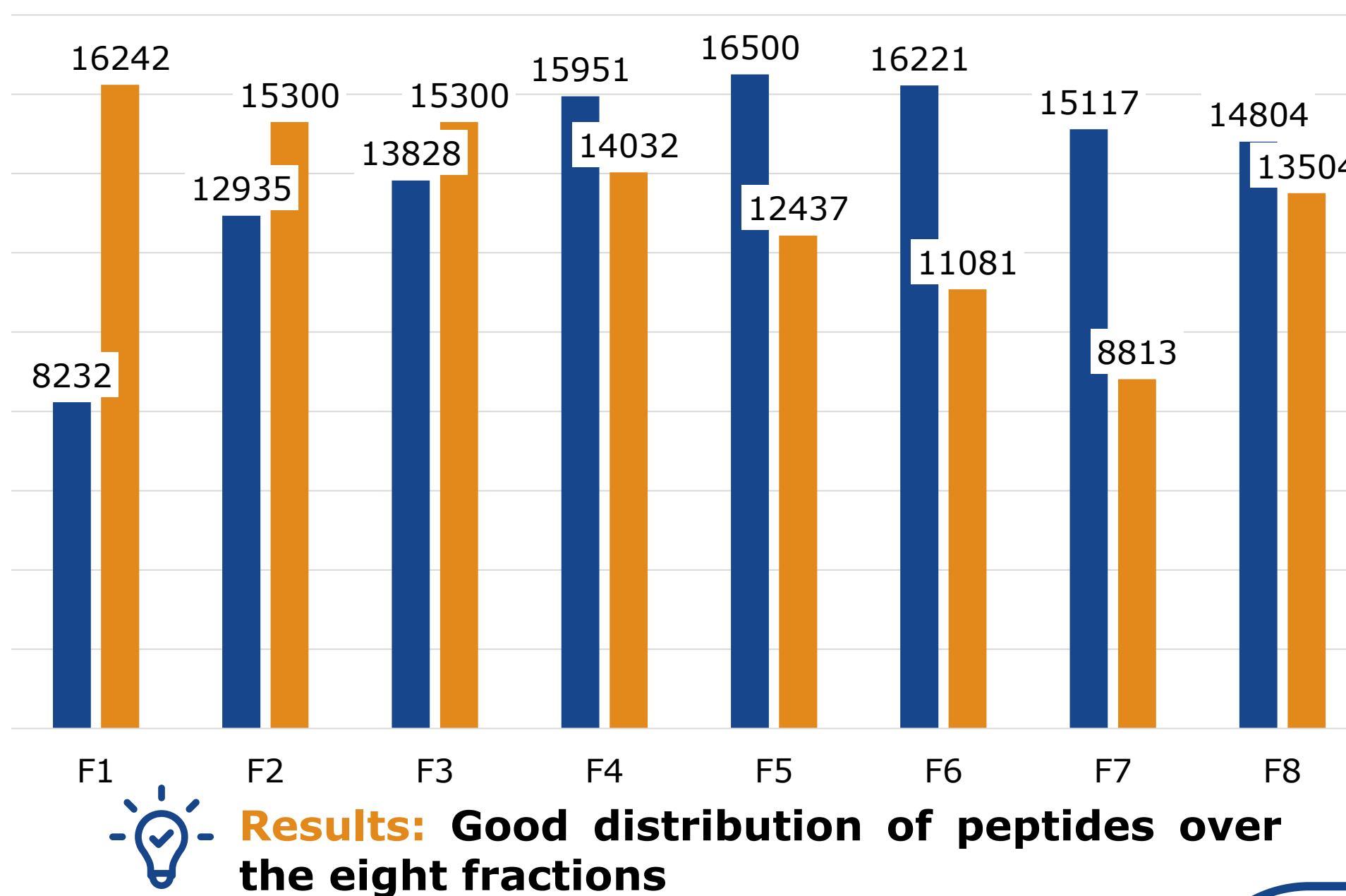


④ Results of peptide fractionation on BioSPE™ PepFrac Tips & comparison with competitor fractionation column

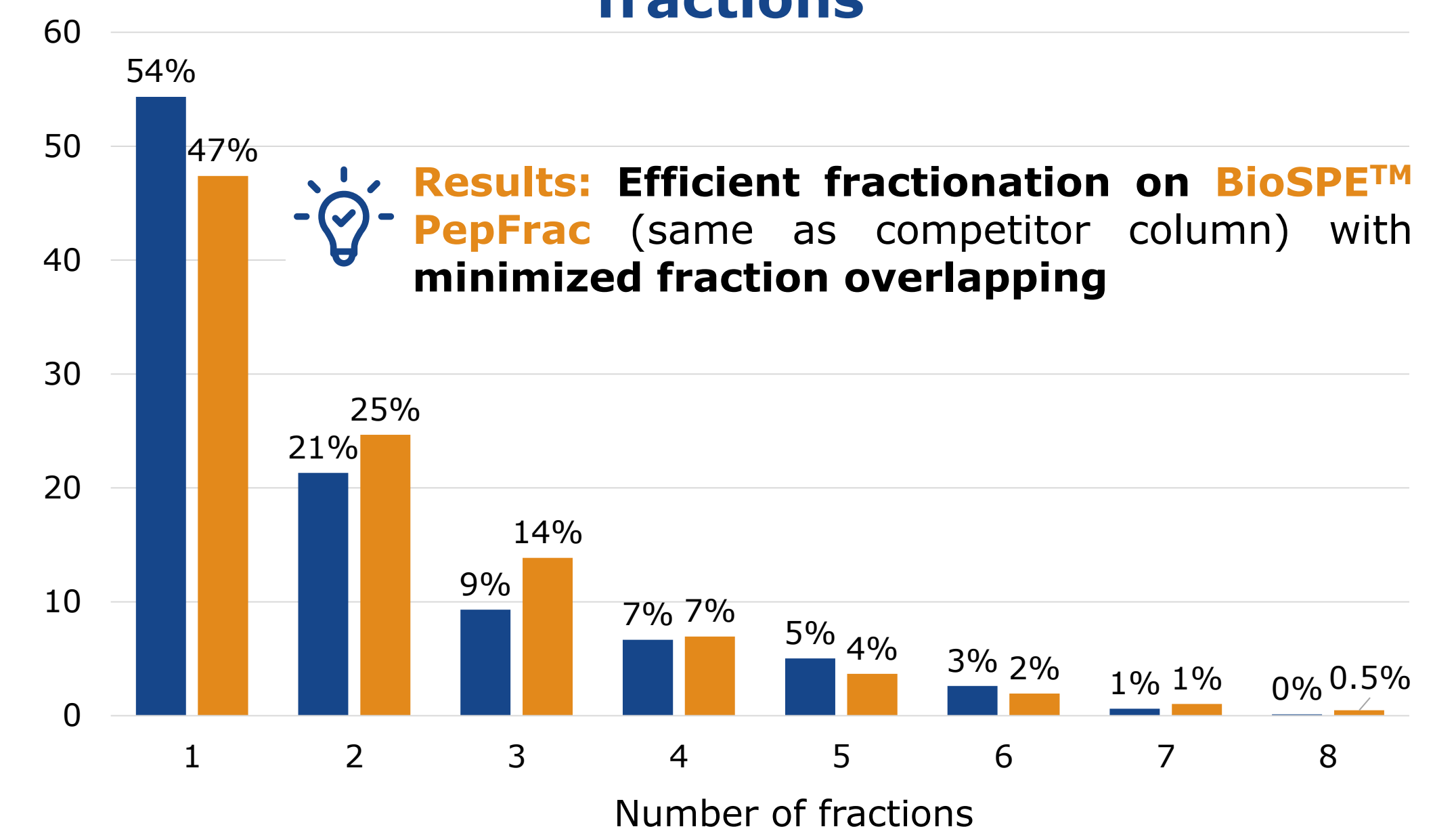
Total number of proteins identified



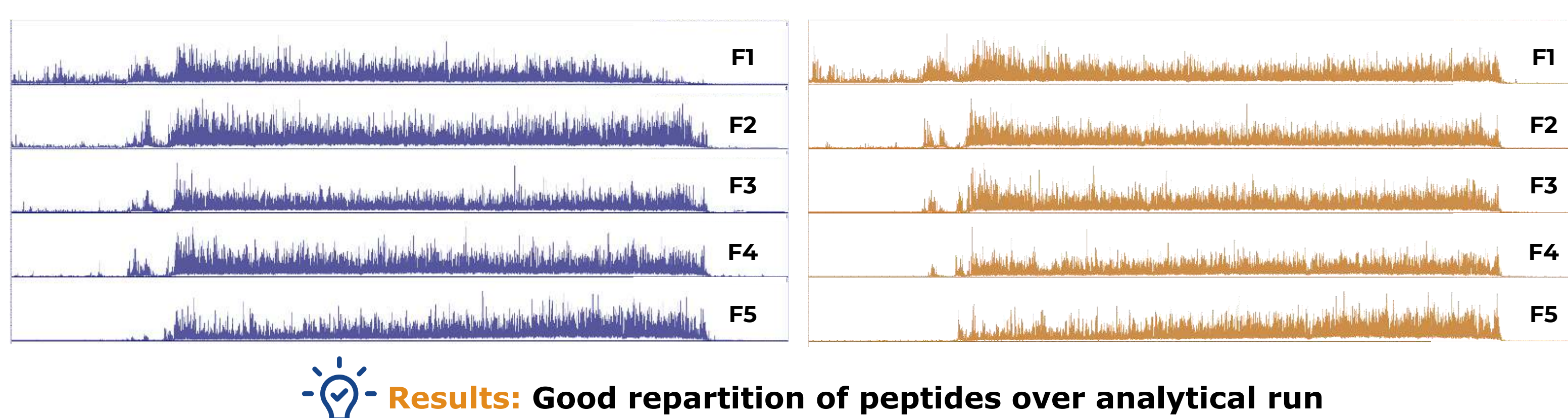
Peptide distribution in each fraction



Percentage of peptides eluting in several fractions



Spectral density over HPLC gradient



Advantages of BioSPE™ PepFrac

- ✓ **No storage constraints for BioSPE™ PepFrac (dry at room temperature for several years)** contrary to competitor column (4°C, in solution)
- ✓ **Time required for evaporation of each fraction almost halved** with BioSPE™ PepFrac Tips
- ✓ **Fractionation of 10 to 100µg 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 20% in the number of proteins identified**, compared to unfractionated samples.