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

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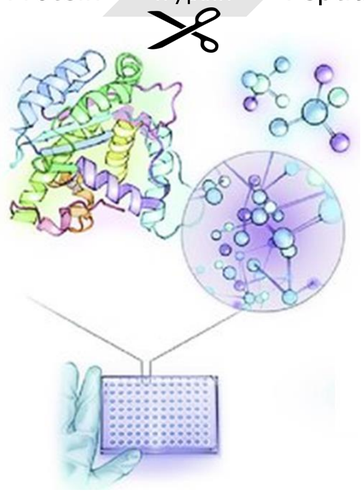
Context: bottom-up proteomics workflow

① Proteolysis

Protein → Trypsin → Peptides



② Reversed-phase peptide fractionation at basic pH

Principle & Advantages

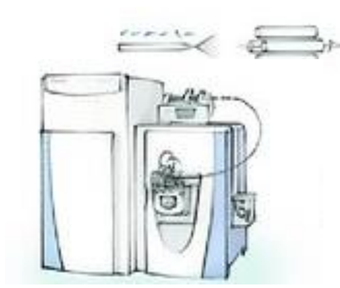
- ✓ **Reduction of sample complexity** for deep proteome sequencing and quantitative analysis
- ✓ **High pH fractionation orthogonal to RPLC peptide separation at low pH**
- ✓ **Increased number of identified proteins** compared to unfractionated peptides
- ✓ **No desalting step required** prior to LC-MS/MS analysis of the fractions

Objective

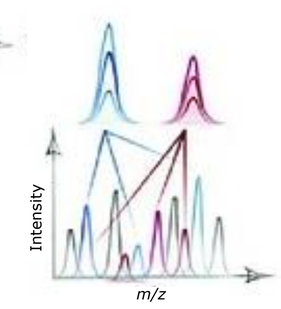
Development of a **simplified procedure for the efficient and fast fractionation of peptides** with a **wide range of properties** (size, polarity and charge)

→ **BioSPE™ PepFrac**

③ LC-MS/MS analysis

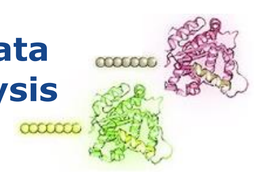


Peptide separation and ionization



Peptide identification and quantification

④ Data analysis



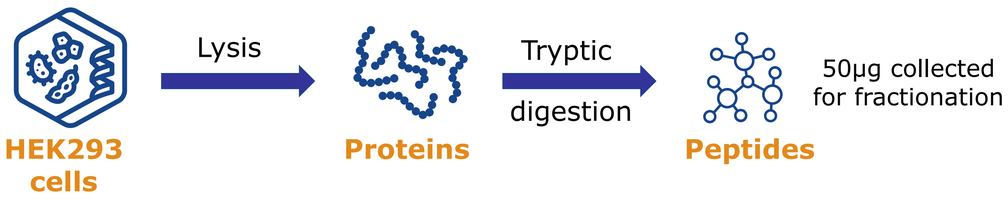
- Protein inference
- PTM identification and localization…

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_2O - 1,500g– 2min	300µL H_2O - 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

NanoLC separation

- **Column:** IonOpticks C18 packed emitter column (25cm x 75µm, 1.6µm)
- **Injected volume:** 1µL
- **Gradient:** 2% to 95% buffer B over 30min at a flow rate of 200 nL/min

  Buffer A: 0.1% FA in water

  Buffer B: 0.1% FA in ACN

MS detection (timsTOF Pro)

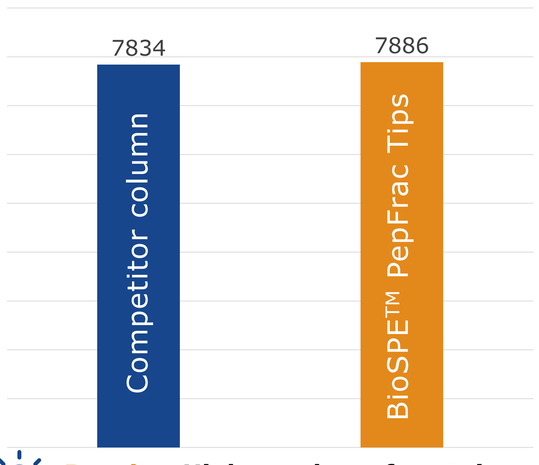
- **Acquisition method:** Parallel Accumulation Serial Fragmentation (PASEF) – Data Dependent Acquisition (DDA)
- ***m/z* range:** 100 to 1700 Th

Data analysis

- **Software:** MaxQuant version 2.0.1.0
- **Database:** UniProtKB/Swiss-Prot *Homo sapiens*

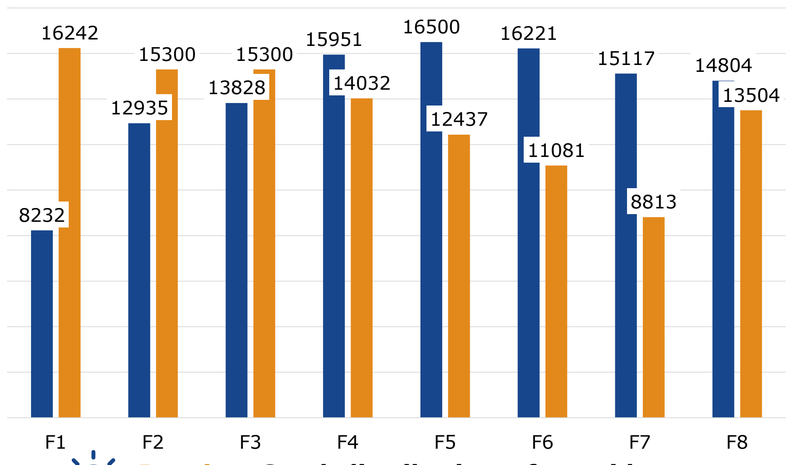
④ Results of peptide fractionation on BioSPE™ PepFrac Tips & comparison with competitor fractionation column

Total number of proteins identified



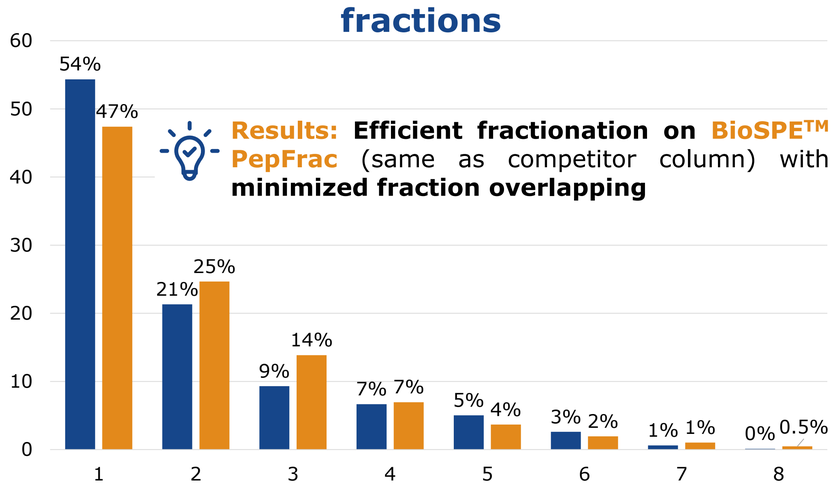
Results: **High number of proteins identified**

Peptide distribution in each fraction



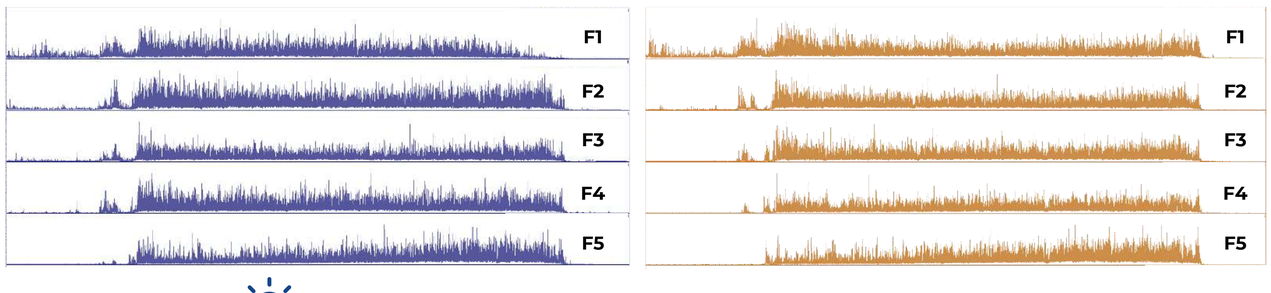
Results: **Good distribution of peptides over the eight fractions**

Percentage of peptides eluting in several fractions



Results: **Efficient fractionation on BioSPE™ PepFrac** (same as competitor column) with **minimized fraction overlapping**

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 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 librairies**, since it leads to an **increase of more than 20% in the number of proteins identified**, compared to unfractionated samples.

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