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R. Maillet, C. Chambon, Thierry Sayd, Arnaud Delavaud, Michel Hébraud,  
Viala Didier

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# Sample preparation for shotgun proteomics: comparison of stacking gel, tube-gel, FASP, S-TRAP, SPE and liquid methods

Robin MAILLET<sup>1</sup>, Christophe CHAMBON<sup>1,2</sup>, Thierry SAYD<sup>1,2</sup>, Arnaud DELAVAUD<sup>3</sup>, Michel HEBRAUD<sup>1,4</sup>, Didier VIALA<sup>1,3</sup>

<sup>1</sup> INRAE, Université Clermont Auvergne, Plateforme d'Exploration du Métabolisme, F-63122 Saint-Genès Champanelle, France.

<sup>2</sup> INRAE, UR QuaPA, F-63122 Saint-Genès Champanelle, France

<sup>3</sup> INRAE, Université Clermont Auvergne, VetAgro Sup, UMRH, 63122, Saint-Genès Champanelle, France

<sup>4</sup> INRAE, Université Clermont Auvergne, UMR MEDis, F-63122 Saint-Genès Champanelle, France

## Objective

- Sample preparation is a crucial step in high-throughput shotgun proteomics, challenged with detergent incompatibility that has a strong influence on the accuracy and robustness of MS analyses. Classical approaches using stacking-gel (SG), Solid Phase Extraction (SPE) or liquid digestion (LD) have been developed but show limitations due to the time-consuming and repetitive sample processing, their recovery efficiency and overall yield. In recent years, strategies by filtration such as filter-aided sample preparation (FASP) based on a molecular weight cut-off (MWCO), and its new alternative, the suspension traps (S-TRAP) confining particulate protein suspensions with the subsequent depletion of interfering substances, have tried to overcome these drawbacks.
- The objective of this work was to compare for the first time all these preparation methods, *i.e.* FASP, S-TRAP, SPE, SG, TG (tube-gel) and LD before subjecting the samples to a label-free semi-quantitative proteomic analysis (shotgun proteomics). A soluble fraction of muscle proteins (100 µg), spiked with 1.5 µg of casein, was used to assess sample preparation methods. Ten replicates were prepared for each method.

## Materials & methods

### Sample preparation

300 mg of pork muscle in 40 mM Tris-HCl, 2 mM EDTA pH 8 buffer

Homogenization - centrifugation

Soluble fraction of muscle proteins (5 mg/ml) spiked with 1,5% casein as internal standard

Control 1D Gel

A soluble fraction of pork muscle proteins was prepared as indicated above. 100 µg were used in 10 replicates for each preparation methods. The quality of sample preparation was checked with a control 1D gel.

20 µl proteins extract 5 µg/µl - - > 100 µg

In-gel methods		In-solution methods		Filtration methods	
SG	TG	LD	SPE	FASP	S-TRAP
Reference	<i>Balliau et al. 2018</i>	Reduction with DTT 2,5 mM 56° C - 30 min	Screening sorbents: C18 (MN), Oasis HLB (Waters), EVOLUTE ABN (Biotage), ISOLUTE EN+ (Biotage), HR-X (MN)	Colgrave et al. 2013	Protein extract in 80 µl ammonium bicarbonate buffer 50 mM
Reduction with DTT 10 mM - 56° C - 30 min	600 ng trypsin	Alkylation with Iodoacetamide 25 mM	SPE ISOLUTE EN+ (Biotage)	Load on a 3kDa millipore tube. Add 100 µl Urea 8 M	Protein extract in 20 µl SDS buffer 10% 100 mM Ammonium bicarbonate pH 7,1
Alkylation with Iodoacetamide 55 mM		Reduction with DTT 2,5 mM 56° C - 30 min	Sample dilution 1:3 (v/v) with 2% FA	Spin 13 000 rpm - 15 min	Reduction with DTT 20 mM
		Alkylation with Iodoacetamide 25 mM	Equilibration 1 ml FA 2%	Reduction with DTT 10 mM	Alkylation with Iodoacetamide 40 mM
		Trypsin digestion	Elution 1 ml MeOH 0,1% TFA	Alkylation with Iodoacetamide 25 mM	Phosphoric acid 12% - 1,2% final
					S-TRAP buffer (90% MeOH, 100 mM ABC pH 7,1)
					Loading 3 x 150 µl - Spin
					Elution 50 µl ABC buffer 50 mM 0,2%FA 80 µl 50% ACN 0,2% FA twice
					2,5 µg trypsin in 150 µl

### Quantitative analysis

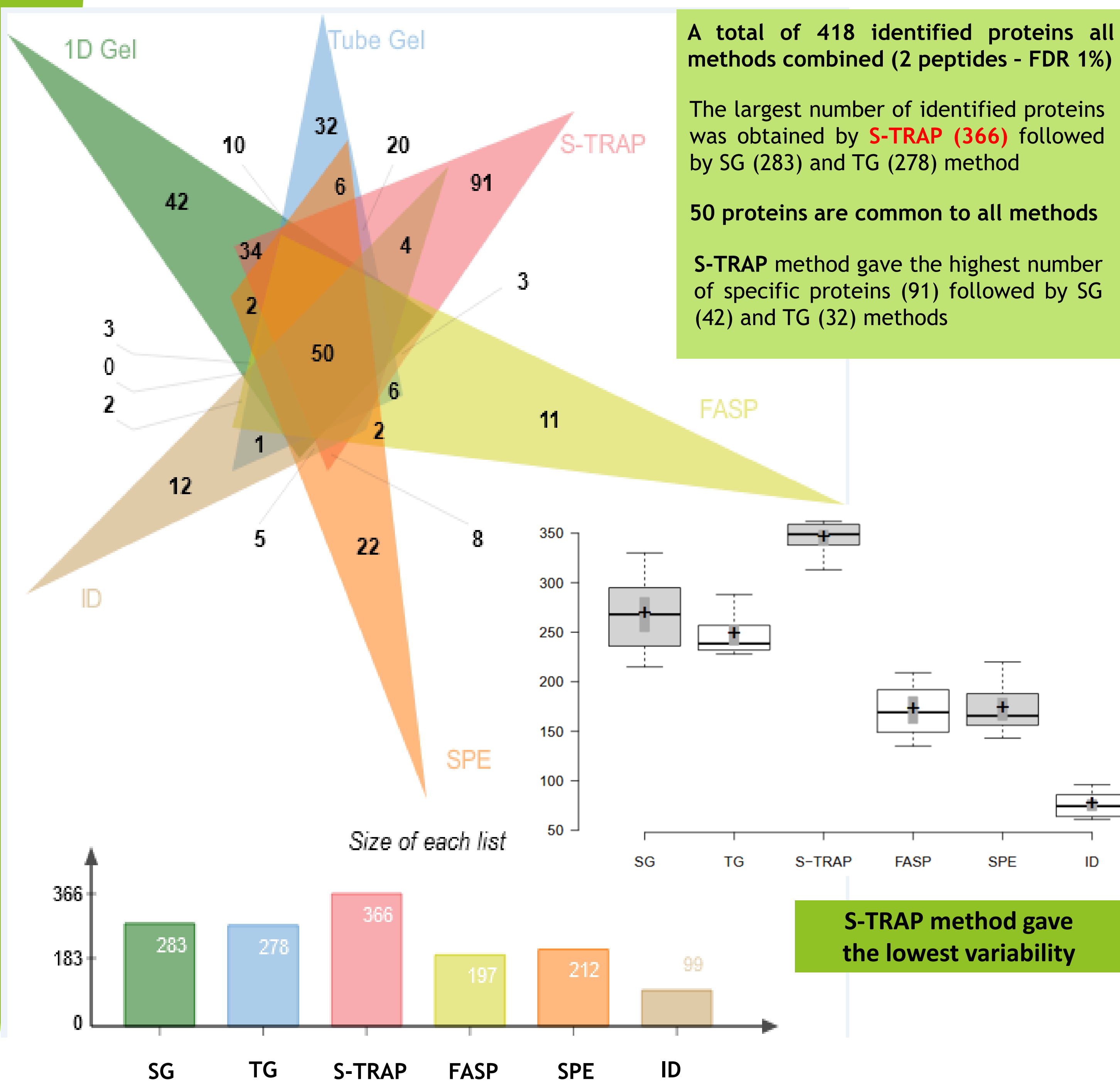
Label-free shotgun by LC-MS/MS and MASCOT identification

Alignment of Ionic maps

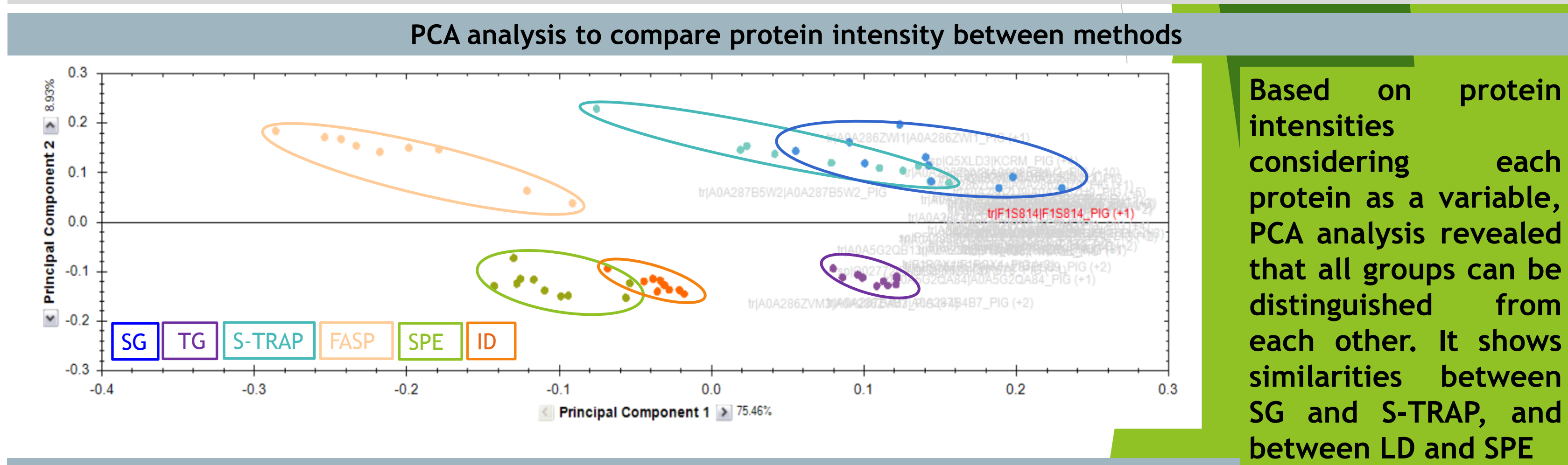
Progenesis Q1 for proteomics

## Results

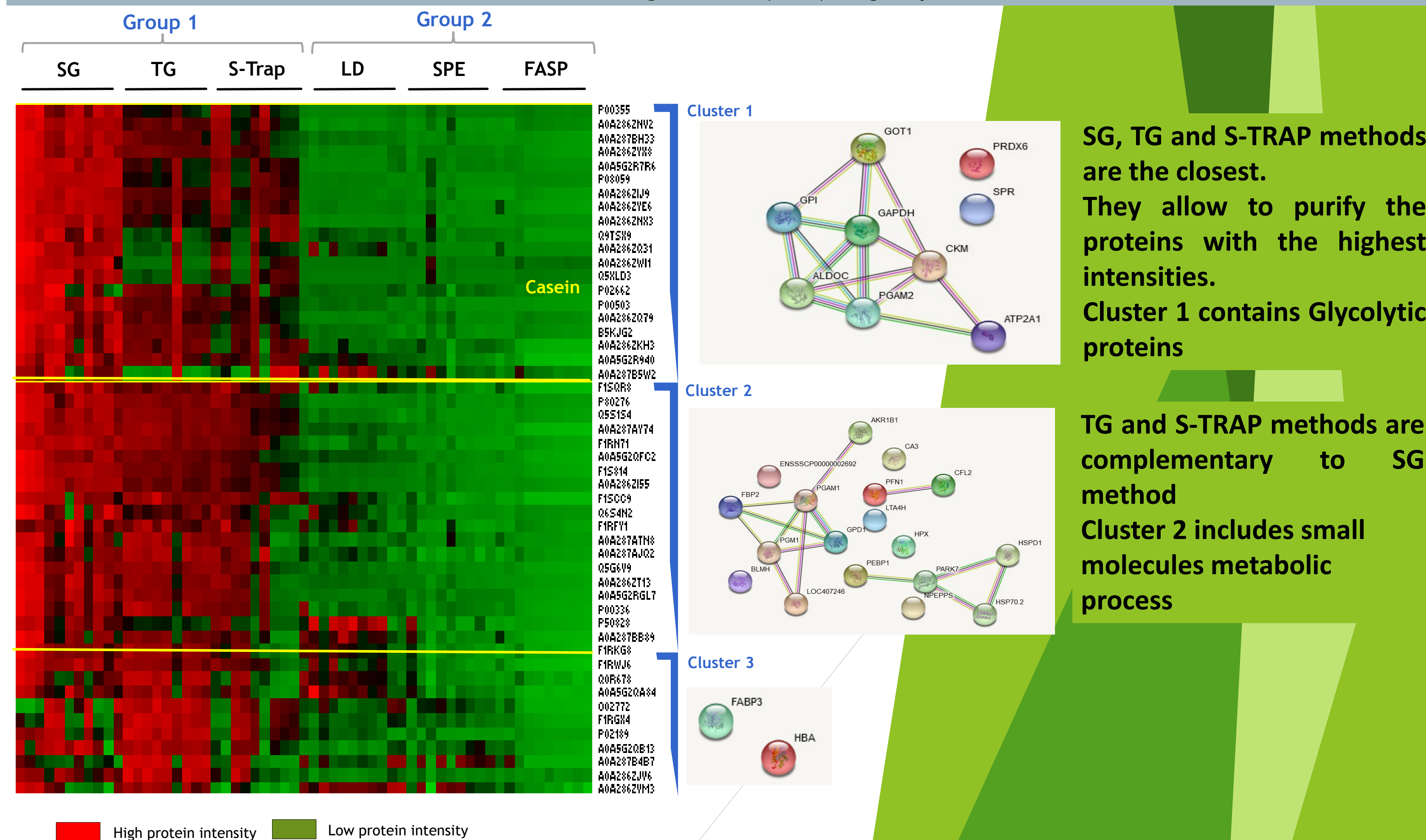
### Identifications



### Statistical analysis : multivariate analysis of the 50 common proteins



### Hierarchical clustering method (HCA) to group methods



## Conclusion

- The originality of this study lay in the comparison of proteins identified by LC-MS/MS from the same sample by implementing several preparation methods based on different principles: gel, liquid and filtration.
- The analysis of the results by Venn diagram, principal-component analysis, hierarchical clustering and the abundance ranking of quantitative proteins highlights significant differences in identified proteins, according to the sample preparation method. Moreover, there is a specificity in the nature of extracted proteins according to the method.
- A total of 418 proteins were identified combining all the methods and the largest number of identified proteins was obtained by S-TRAP (366), followed by SG (283) and TG (278) methods.
- Statistical results and the qualitative analyses of significant proteins indicate that S-TRAP method outperforms SG method.
- S-TRAP would purify the majority of the proteins in a sample rapidly and with the greatest intensity.
- The faster and easier S-TRAP method turns out to be the best alternative to replace classical in-gel and in-solution methods, resulting in an ultrafast sample-preparation approach for shotgun proteomics.