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

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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.

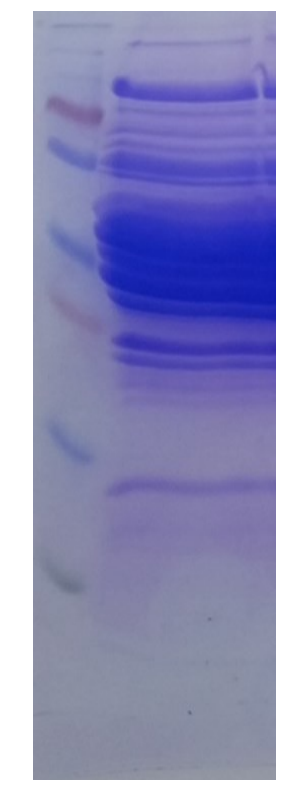
Materials & methods

Sample preparation

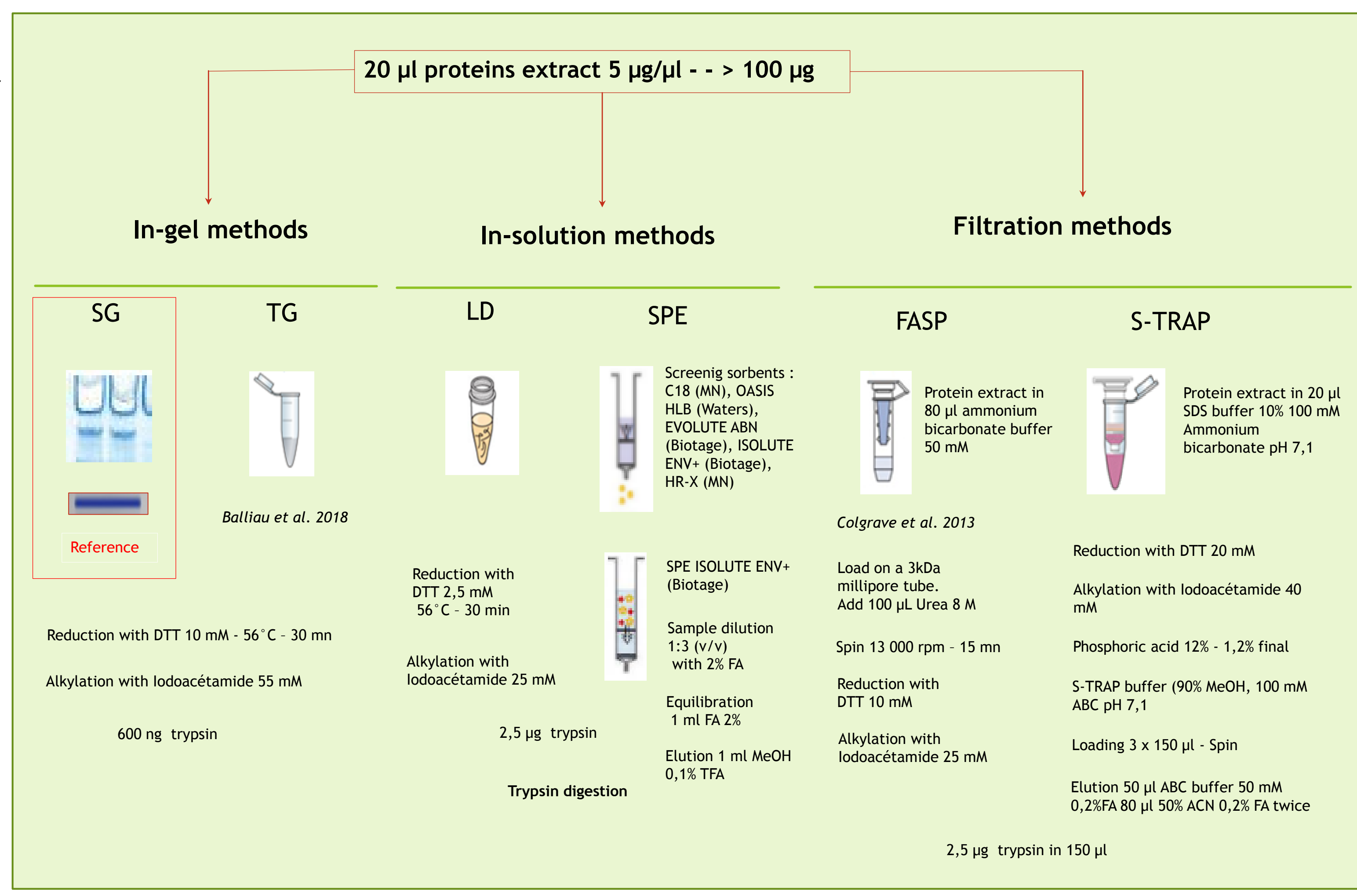
Quantitative analysis

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

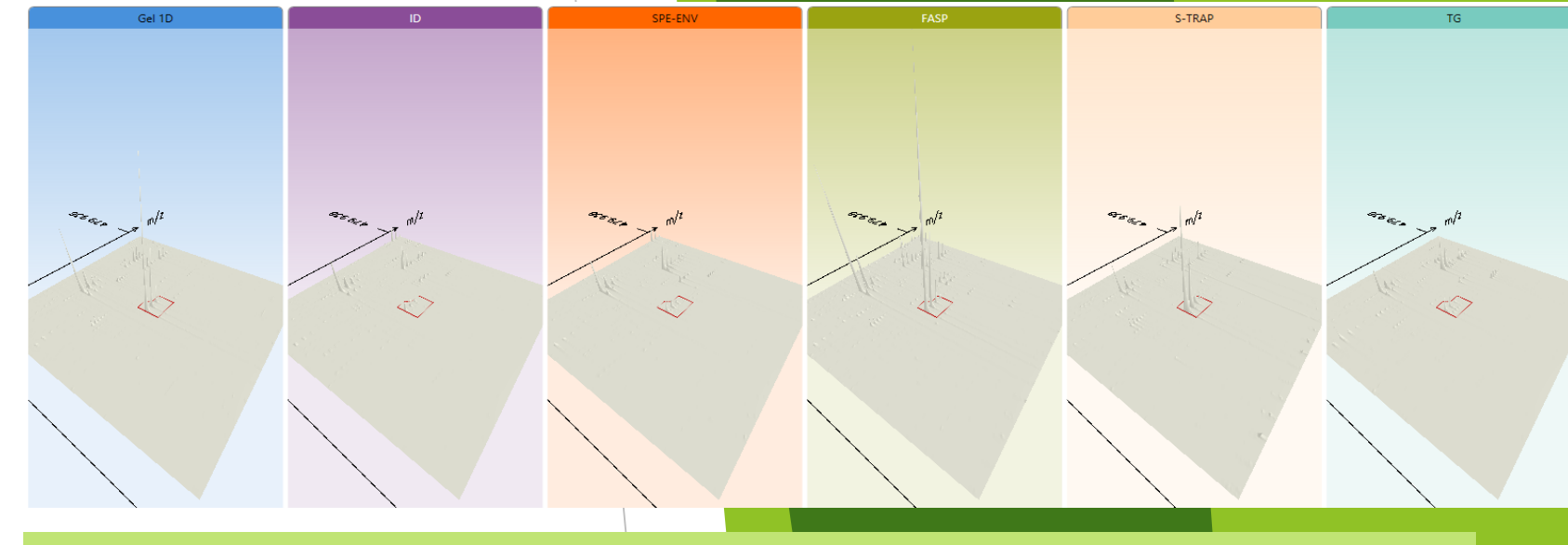


A soluble fraction of pork muscle proteins was prepared as indicated above. 100 µg were used in 10 replicates for each preparation method. The quality of sample preparation was checked with a control 1D gel.



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

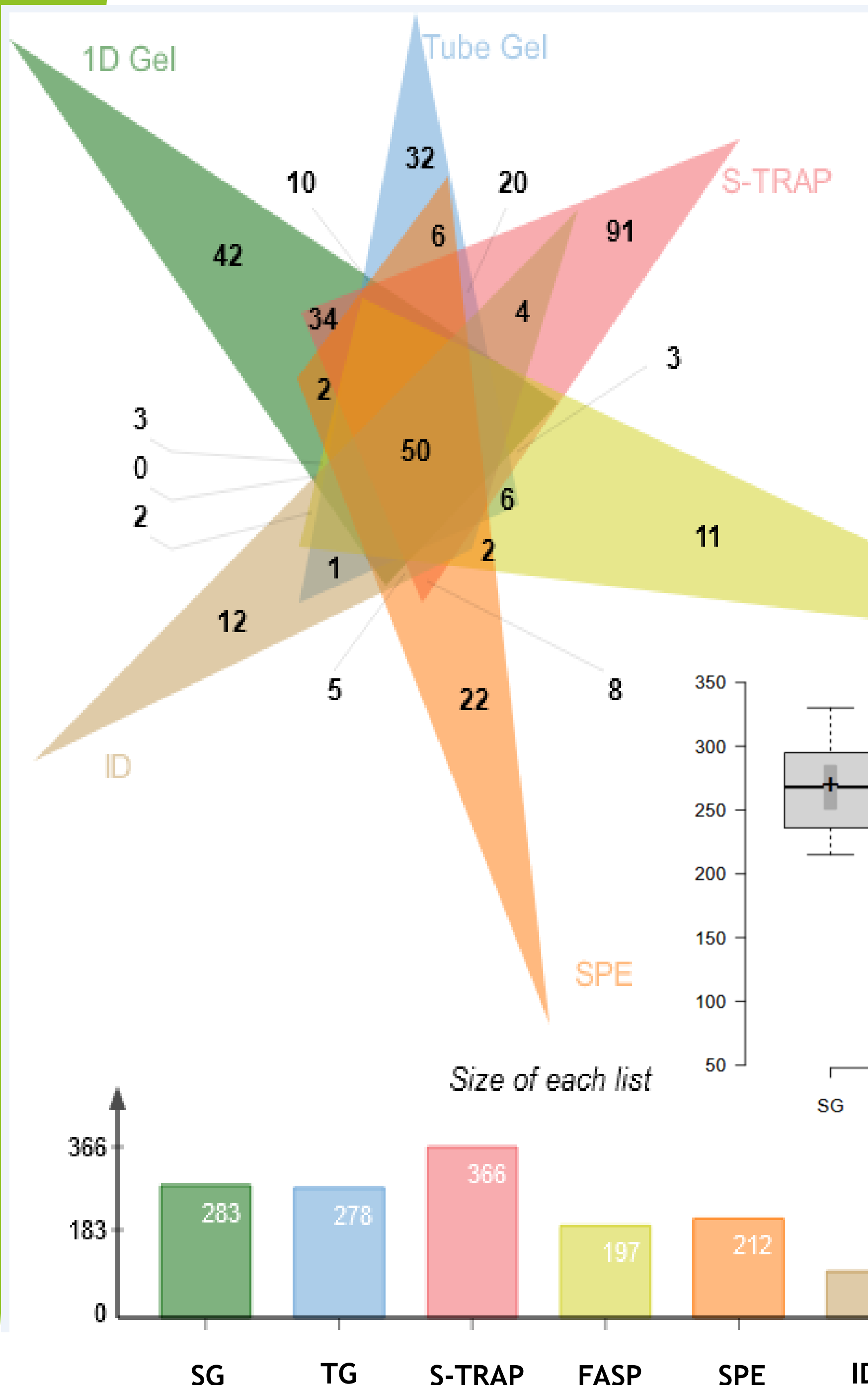
Alignment of Ionic maps Progenesis Q1



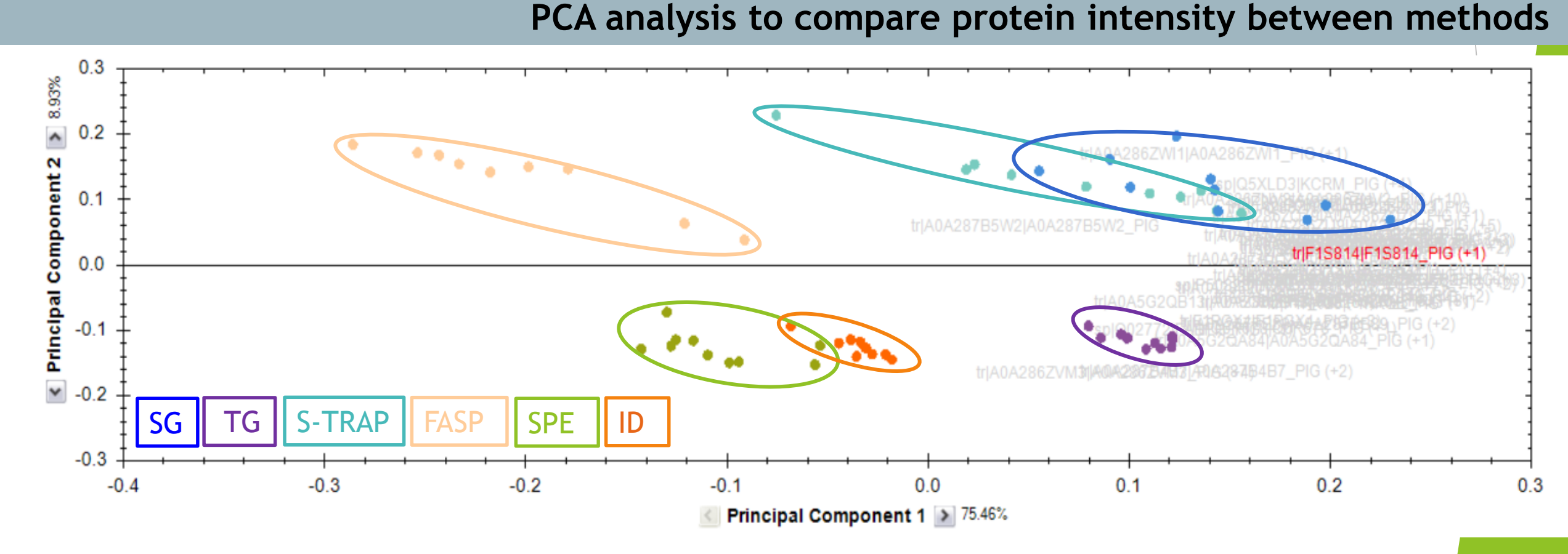
Results

Identifications

Statistical analysis : multivariate analysis of the 50 common proteins

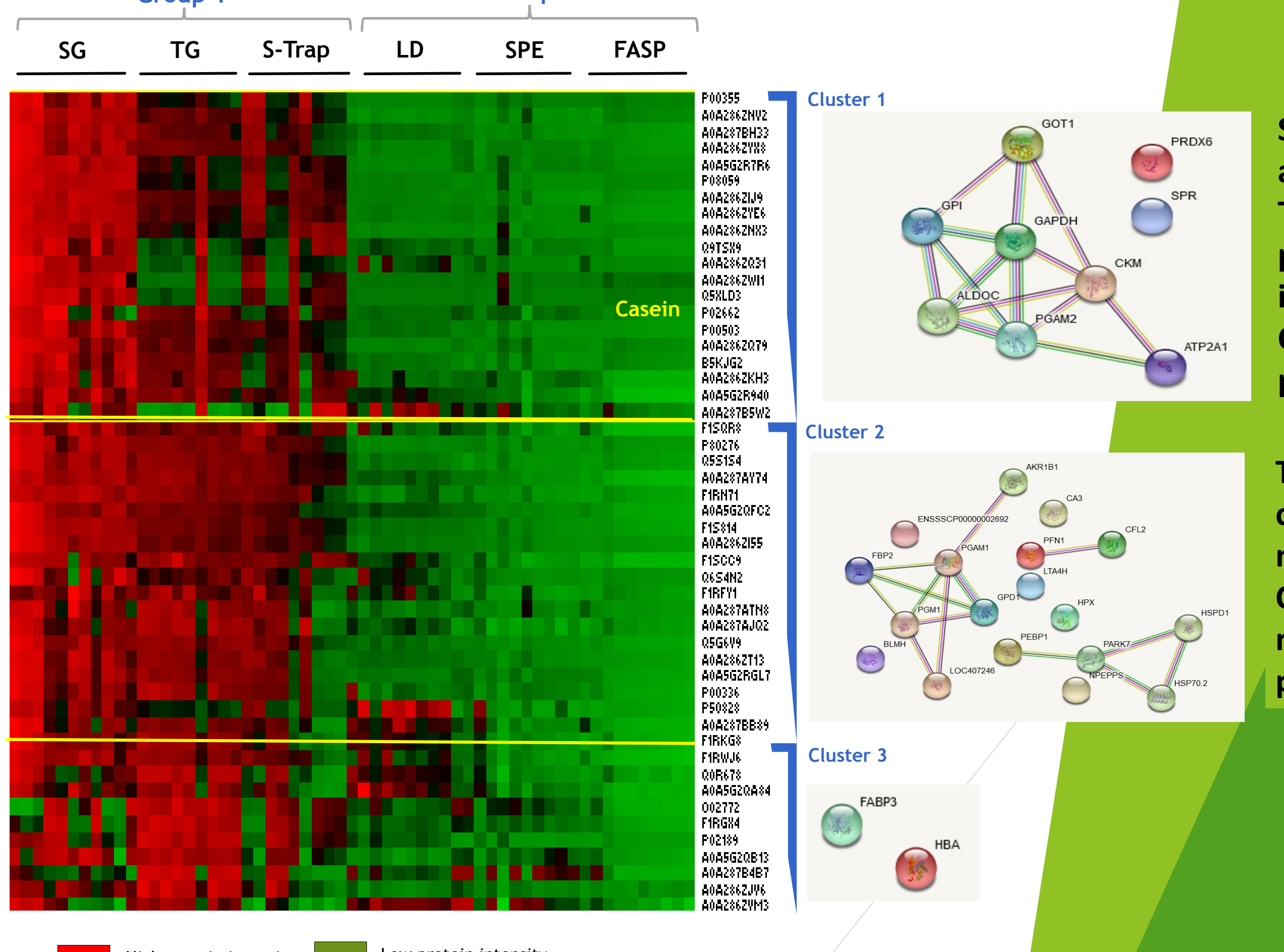


A total of 418 identified proteins all methods combined (2 peptides - FDR 1%)
The largest number of identified proteins was obtained by S-TRAP (366) followed by SG (283) and TG (278) method
50 proteins are common to all methods
S-TRAP method gave the highest number of specific proteins (91) followed by SG (42) and TG (32) methods



Based on protein intensities considering each protein as a variable, PCA analysis revealed that all groups can be distinguished from each other. It shows similarities between SG and S-TRAP, and between LD and SPE

Hierarchical clustering method (HCA) to group methods



SG, TG and S-TRAP methods are the closest. They allow to purify the proteins with the highest intensities. Cluster 1 contains Glycolytic proteins
TG and S-TRAP methods are complementary to SG method. Cluster 2 includes small molecules metabolic process

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.