HIGH SENSITIVITY ANALYTICAL PLATFORMS FOR THE CHARACTERIZATION OF PROTEINS AND GLYCOPROTEINS BY HIGH PERFORMANCE SEPARATION TECHNIQUES COUPLED TO MASS SPECTROMETRY.

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Introduction

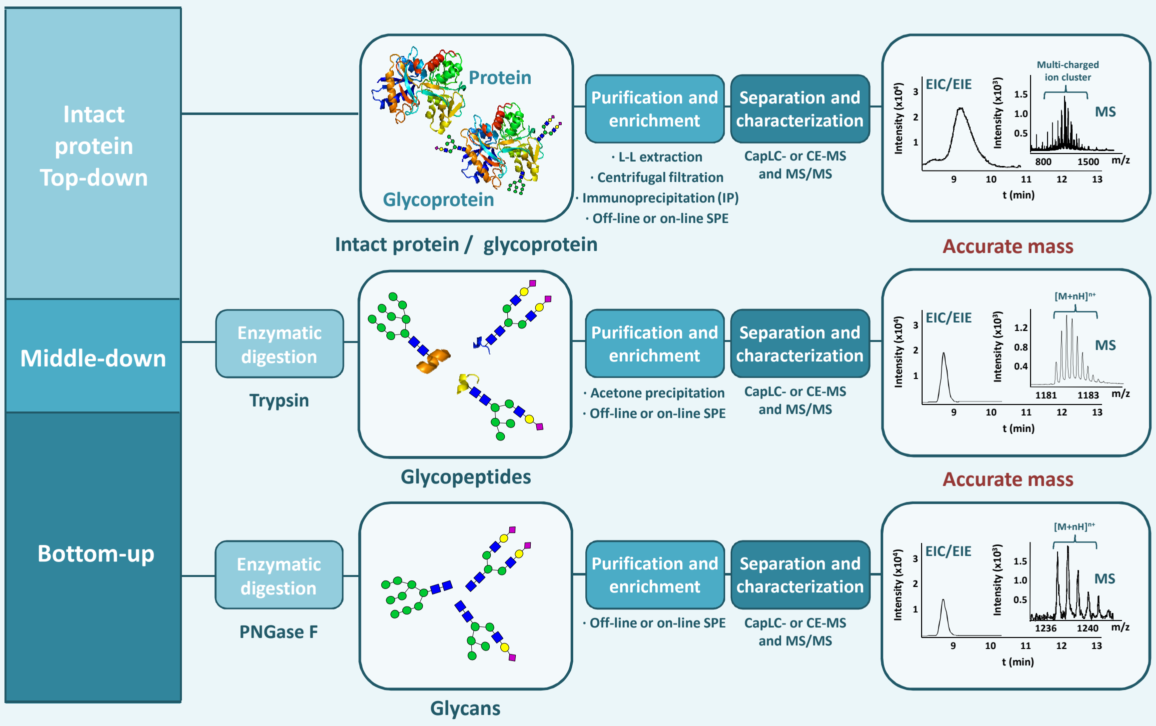
During the last years, there is a growing interest in the application of proteomics and glycoproteomics for research of new clinical biomarkers. A significant part of these studies is focused on the development of novel high sensitivity analytical platforms for the purification, preconcentration, separation, identification and characterization of intact proteins and glycoproteins, peptides, glycopeptides and glycans in biological samples to improve prevention, diagnosis, prognosis, follow-up and therapeutic treatment of different diseases.

Electrospray ionization mass spectrometry (MS) coupled to high performance separation techniques, such as nano or capillary liquid chromatography and capillary electrophoresis (nanoLC-MS, CapLC-MS and CE-MS) are nowadays widely accepted by the scientific community in proteomics research, because of the unbeatable advantages for the separation and characterization of the analytes of interest from small amounts of complex biological samples, using top-down, middle-down and bottom-up strategies [1,2]. However, there are still many analytical challenges related to the structural microheterogeneity of proteins and glycoproteins, the wide dynamic range of protein concentrations, the sample matrix complexity and the detection sensitivity. In particular, these three last issues are closely related with selection of an appropriate sample pretreatment.

Over the last years, considerable efforts have been made to develop selective and sensitive sample pretreatment methods for the rapid, simple, reproducible and high-throughput purification and preconcentration of protein and glycoprotein biomarkers. Here we will present an overview of different innovative methods that we have developed to be applied off-line or on-line before CE-MS and CapLC-MS, for the analysis of intact proteins and glycoproteins, peptides, glycopeptides and glycans in biological samples [3,4].

Experimental

Proteomics and glycoproteomics strategies

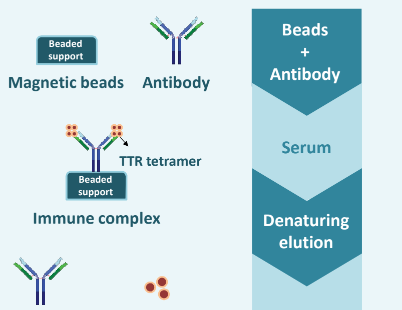


Results

Analysis of intact human transthyretin (TTR)

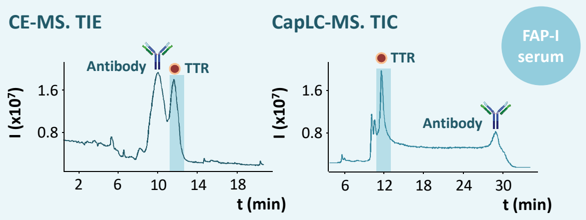
Reference [1]

1. Immunoprecipitation (IP)

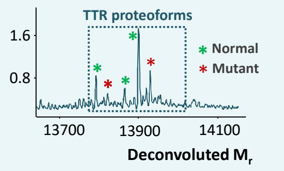


Application to familial amyloidotic polineuropathy type I (FAP-I)

2. Analysis by CE-MS and CapLC-MS



TTR deconvoluted MS



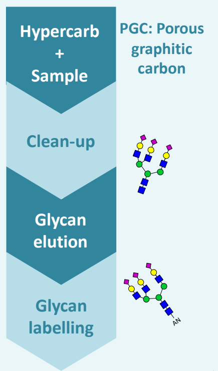
The off-line IP allows the selective purification of normal and mutant TTR proteoforms

CE-MS and CapLC-MS show excellent performance for the diagnosis of FAP-I

Glycan analysis of human α-1-acid glycoprotein (hAGP)

Reference [2]

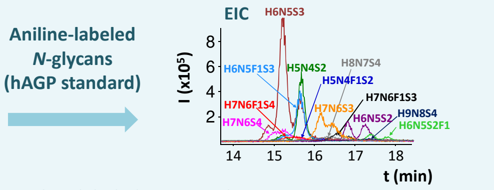
1. Off-line SPE



Glycan relative quantitation (Control vs. Pathological)

2. Analysis by CapZIC-HILIC-MS

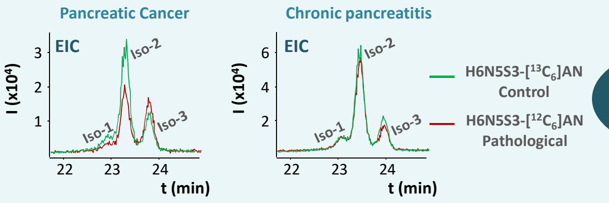
Aniline-labeled *N*-glycans (hAGP standard)



The off-line Hypercarb method allows the selective purification of hAGP N-glycans

CapZIC-HILIC-MS shows excellent performance for the diagnosis of pancreatic diseases

Aniline-labeled H6N5S3 glycan



Glycan isomer biomarker

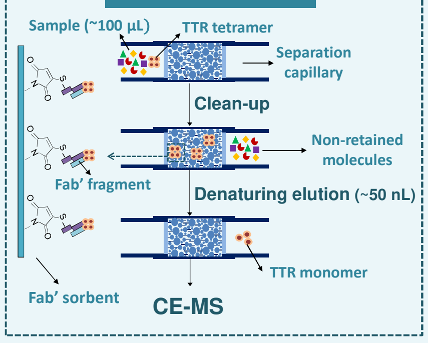
Analysis of intact human transthyretin (TTR)

References [3,4]

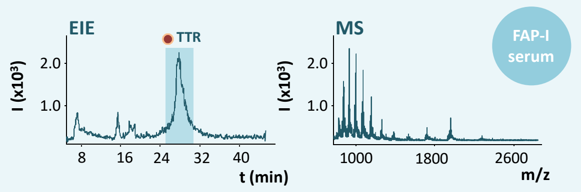
On-line solid-phase extraction capillary electrophoresis (SPE-CE)

- **Conventional chromatographic sorbents**: reversed-phase, polymeric, etc.
- **Immunoaffinity sorbents**: intact antibodies, **Fab' antibody fragments**, etc.

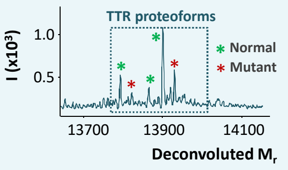
Fab'-IA-SPE-CE-MS



Application to familial amyloidotic polineuropathy type I (FAP-I)



TTR deconvoluted MS



LODs with Fab'-IA-SPE-CE-MS are ~50-fold lower than by CE-MS

Fab'-IA-SPE-CE-MS shows excellent performance for the diagnosis of FAP-I

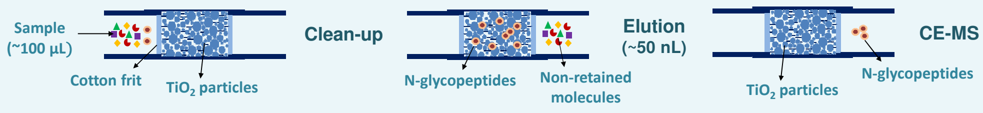
Glycopeptide analysis of recombinant human erythropoietin (rhEPO)

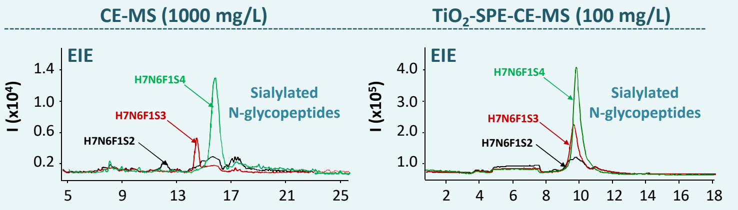
Reference [5]

rhEPO glycoprotein ⟶ Enzymatic digestion ⟶ **Glycopeptides**

- N_{83} glycopeptides
- O_{126} glycopeptides

N_{83}-glycopeptide enrichment by TiO_2-SPE-CE-MS





LODs with TiO_2-SPE-CE-MS are ~100-fold lower than by CE-MS

TiO_2-SPE-CE-MS shows high selectivity towards sialylated N-glycopeptides from rhEPO

Conclusions

- We develop novel high sensitivity analytical platforms for the analysis of intact proteins, glycopeptides and glycans in biological samples.
- Off-line and on-line sample pretreatments before CE-MS, CapLC-MS and nanoLC-MS are necessary for the simple, reproducible and high-throughput purification and preconcentration of protein and glycoprotein biomarkers.
- Combination of intact protein analysis and the rest of strategies is necessary for the accurate and comprehensive protein and glycoprotein characterization.

References

[1] Pont L., Poturcu K., Benavente F., Barbosa J., Sanz-Nebot V., J. Chromatogr. A (2016), 1444, 145-153.
[2] Mancera-Arteu M., Giménez E., Barbosa J., Sanz-Nebot V., Anal. Chim. Acta (2016), 940, 92-103.
[3] Pont L., Benavente F., Barbosa J., Sanz-Nebot V., Talanta (2017), 170, 224-232.
[4] Benavente F., Medina-Casanellas S., Giménez E., Sanz-Nebot V., Methods Mol. Biol. (2016), 1466, 67-84.
[5] Giménez E., Ramos-Hernan R., Benavente F., Barbosa J., Sanz-Nebot V., Anal. Chim. Acta (2012), 709, 81-90.