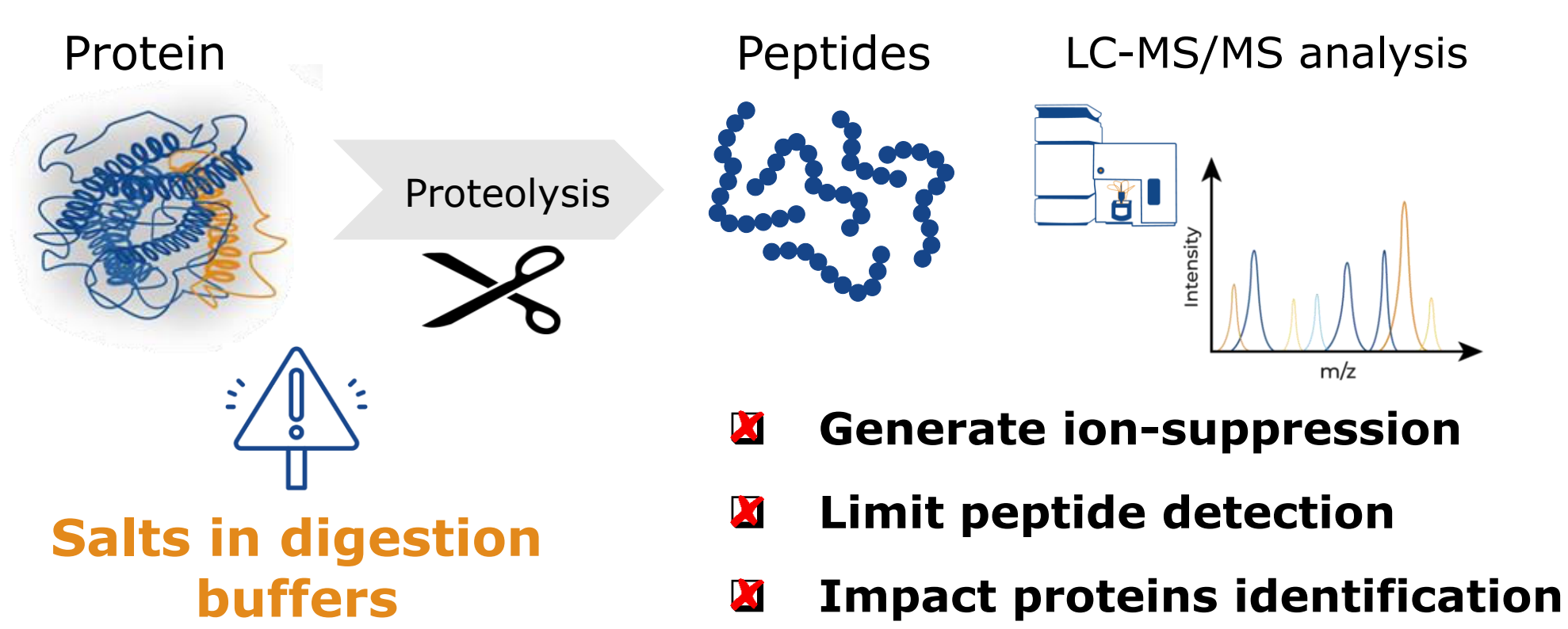


Development and optimization of a method for automated peptide desalting on the DigestPro MSi robot using AttractSPE® Tips C18

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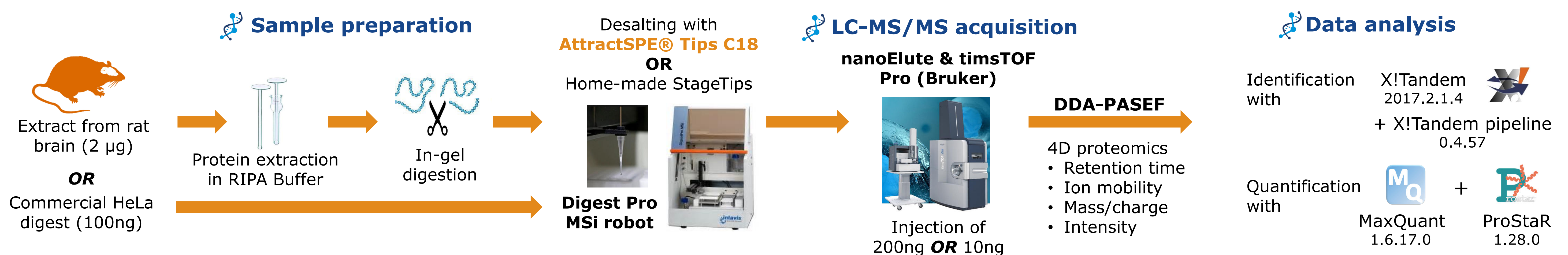
Introduction



Peptide desalting is a crucial step of sample preparation to perform bottom-up approach in proteomics analysis by LC-MS/MS. Throughout this study, **AttractSPE® Tips C18**, packed with a membrane made of small sorbent particles tightly embedded in a monolithic disk to combine **high capacity** and **small dead volume**, were **evaluated and compared to home-made StageTips** (with C18 SPE disks) for the **desalting of 2µg and 100ng of protein digest** using the **DigestPro MSi robot** (CEM). The program on the robot was also optimized to **reduce the desalting time per sample**.



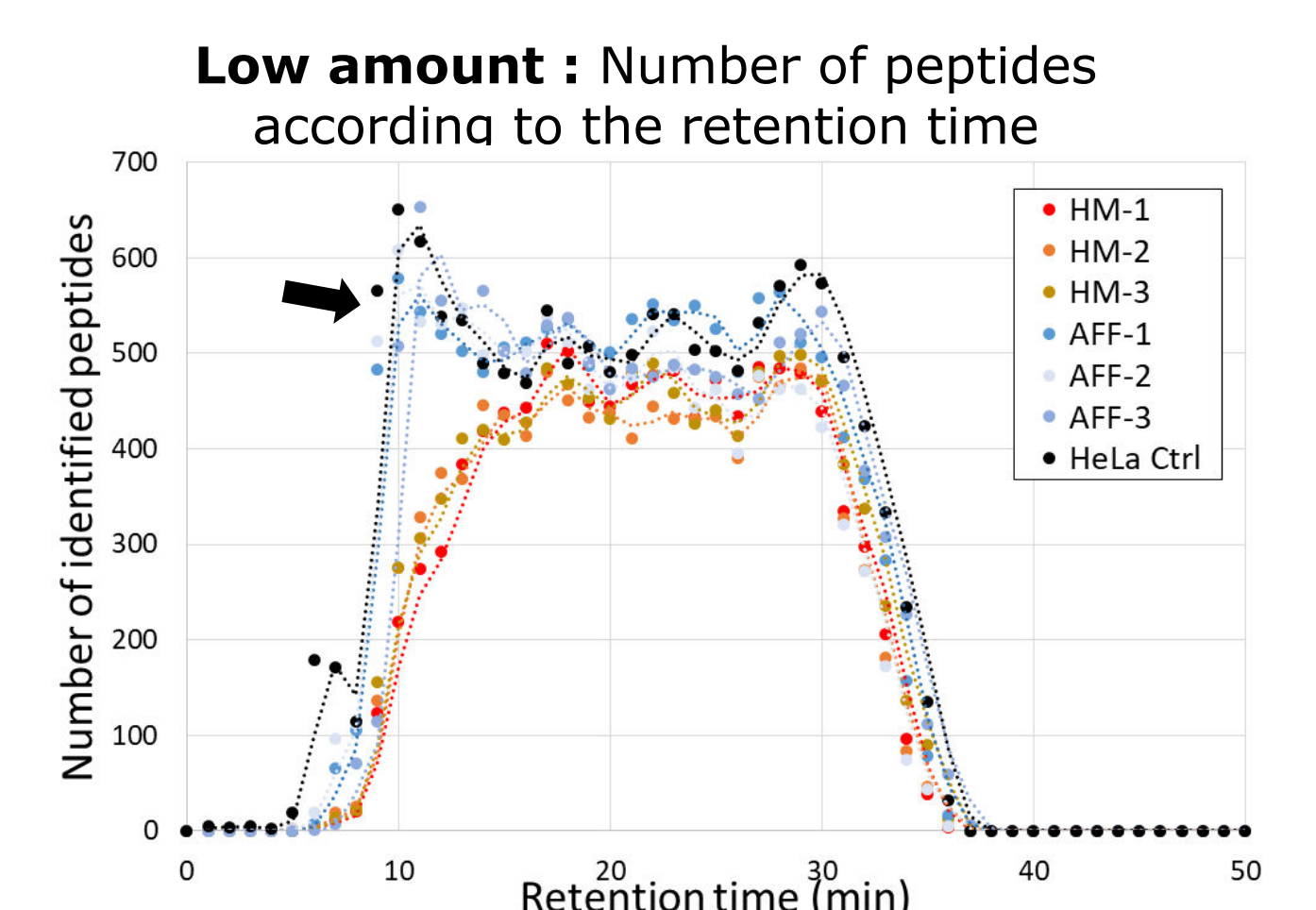
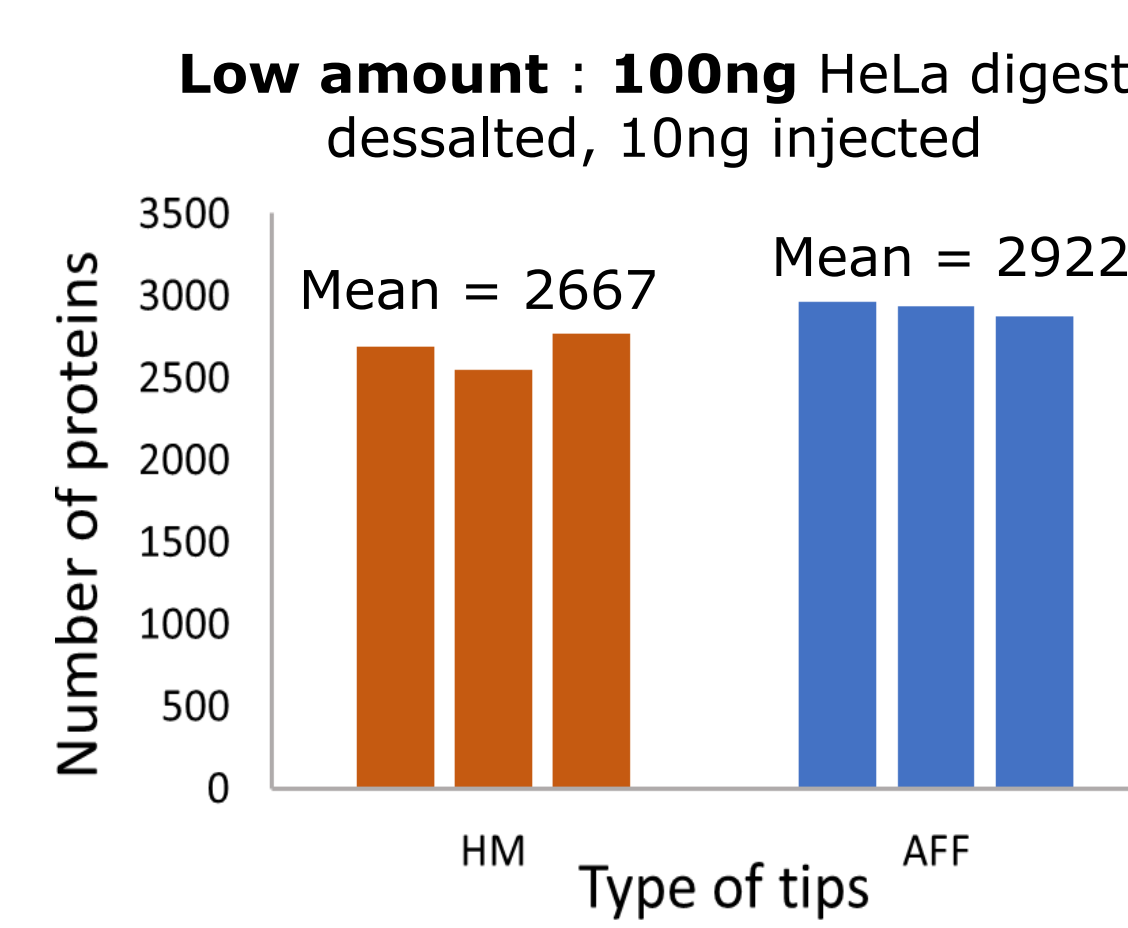
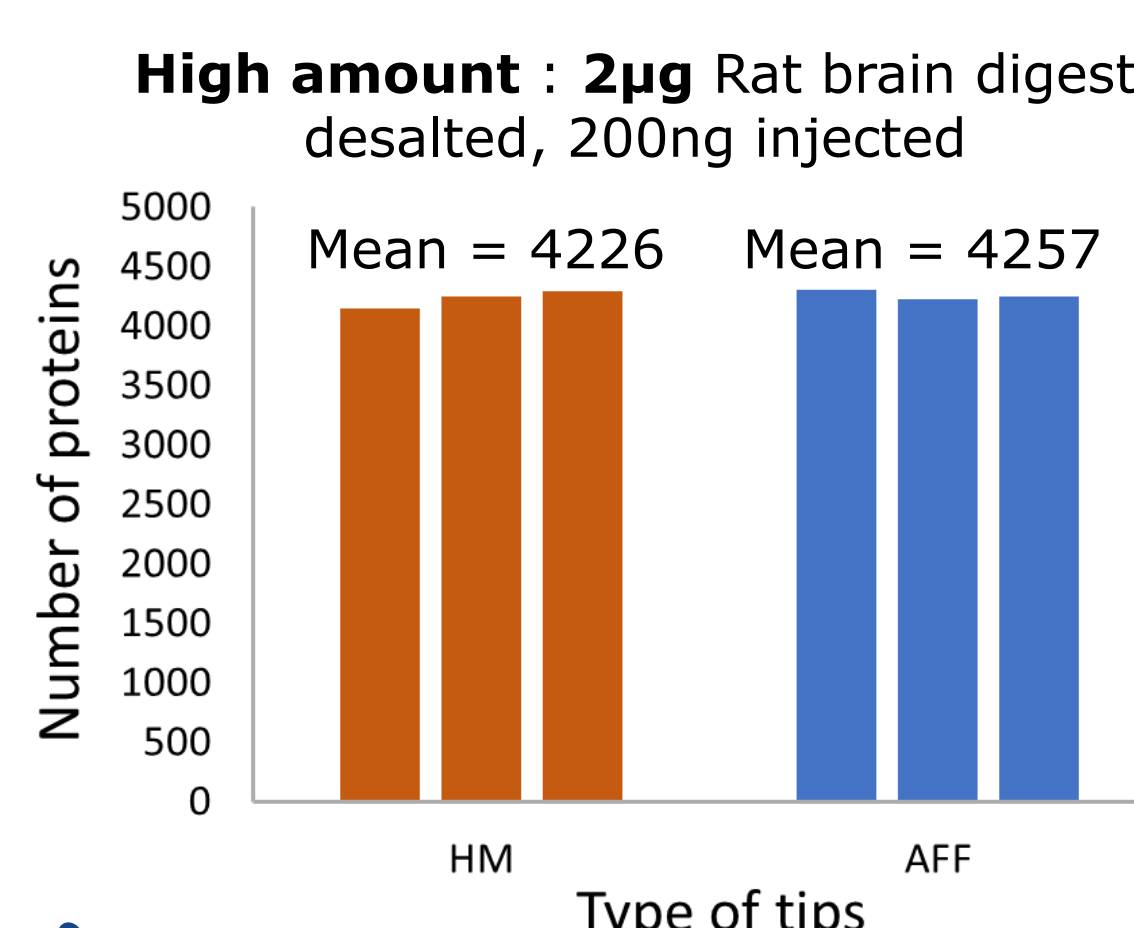
Methods



Identification

High amount samples of rat brain digest (2µg) desalted with **AttractSPE® Tips C18 (AFF)** in triplicate shows **similar number of identified proteins** compared to home-made StageTips (HM). **With low amount of HeLa digest (100ng), AttractSPE® Tips C18 give 9 % average gain compared to HM.**

AttractSPE® Tips C18 and HM show similar TIC profiles with 2µg of digest while **AttractSPE® Tips C18 show a better retention of hydrophilic peptides than HM with 100ng of digest.**

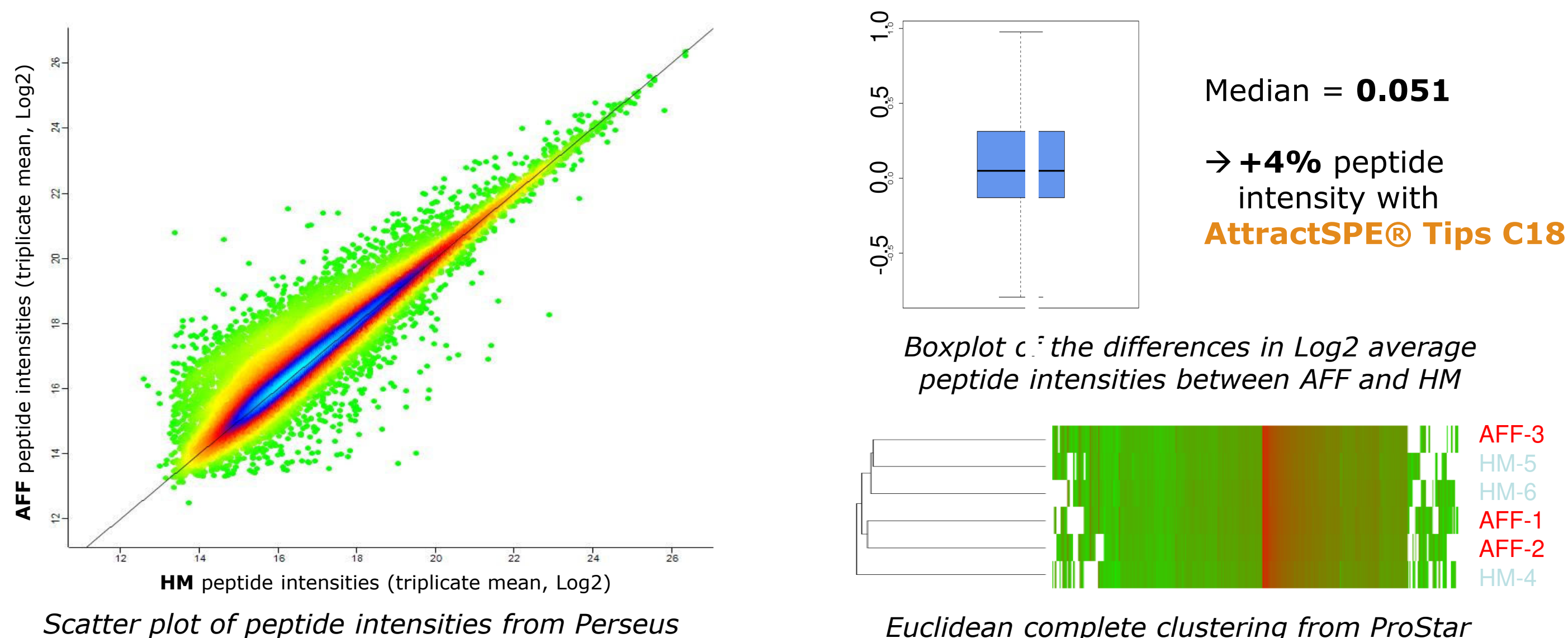


AttractSPE® Tips C18 presents a wider spectrum of interactions including hydrophilic peptides

Quantification

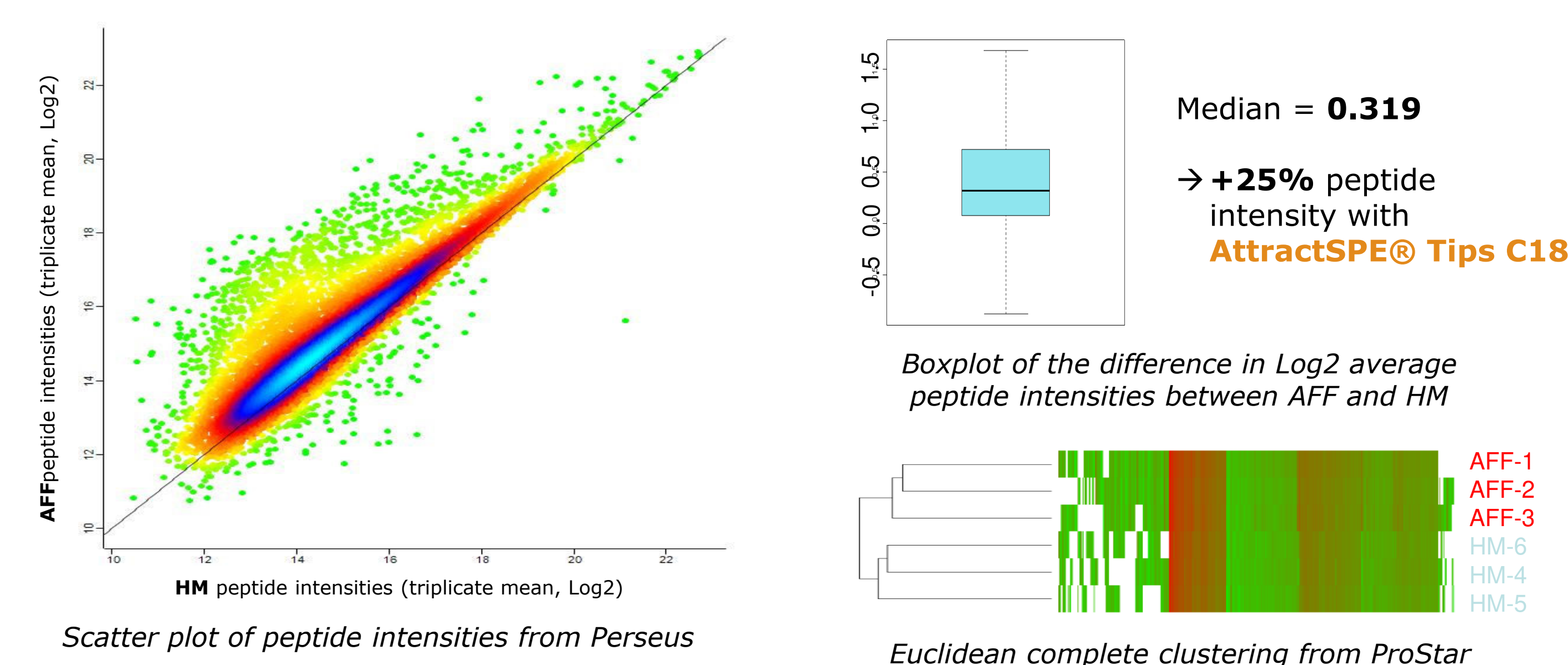
Comparison between AttractSPE® Tips C18 and home-made StageTips

High amount : Peptides LFQ intensities of 200ng Rat brain Digest (2µg desalted)



Almost **no difference** observed in terms of peptide intensities **when desalting high quantities (2µg)** with **AttractSPE® Tips C18** or HM Tips.

Low amount : Peptides LFQ intensities of 10ng HeLa Digest (100ng desalted)



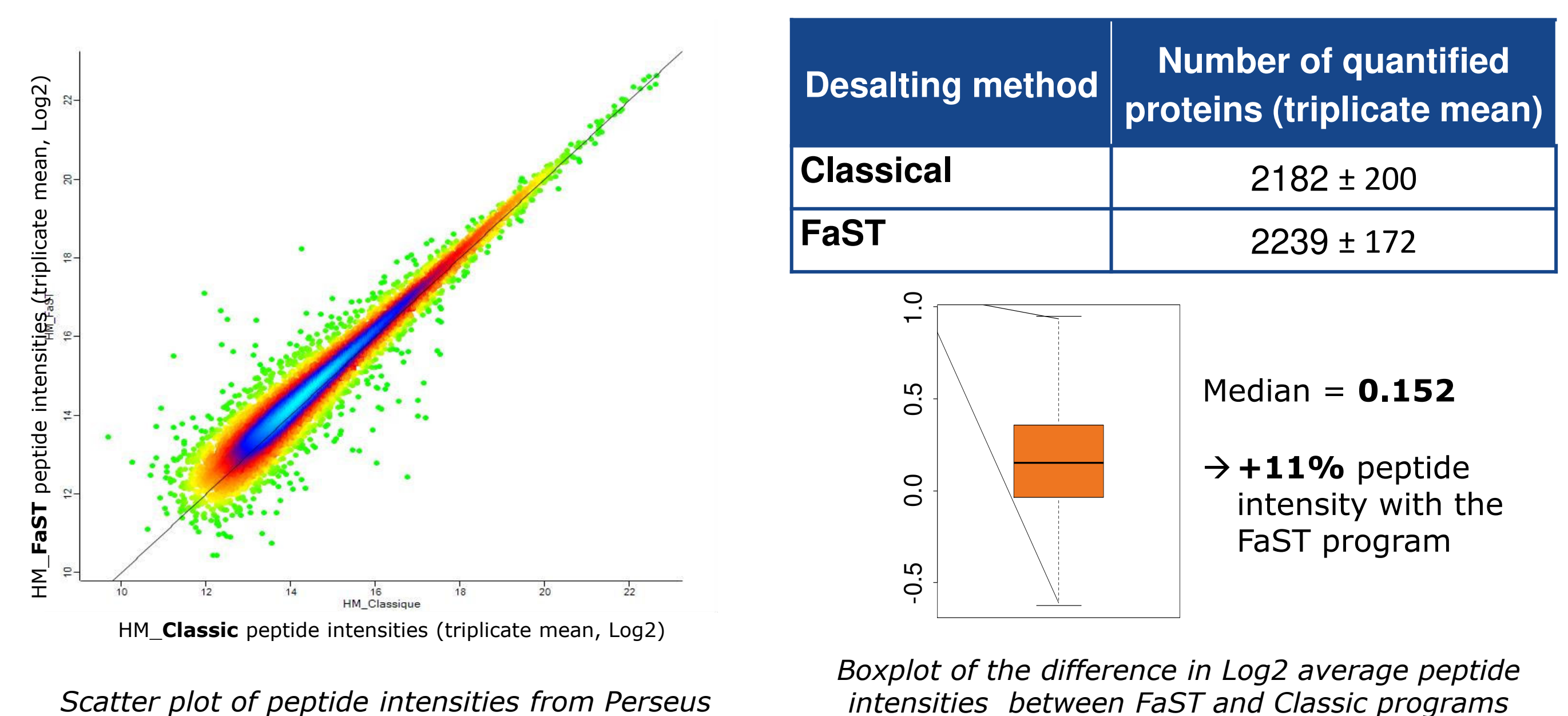
When working **with low amount of digest, AttractSPE® Tips C18 give 25% gain in peptide intensities** compared to HM. Moreover, **AttractSPE® Tips C18** and HM separate in two distinct groups on the clustering, with less missing values in the **AttractSPE® Tips C18** group.

Optimization of the desalting program

Steps	Classic method (40min/sample)	FaST method (10min/sample)
Conditioning: dispense speed		
200µL MeOH	0.1mL/min	0.2mL/min
100µL 50% ACN 0.5% acetic acid		
200µL 0.5% acetic acid		
Sample load: dispense speed		
68µL 0.5% acetic acid	0.01mL/min	0.05mL/min
Sample wash: dispense speed		
100µL 0.5% acetic acid	0.1mL/min	0.2mL/min
Elution: dispense speed		
80µL 0.5% acetic acid	0.01mL/min	0.1mL/min
Elution: volumes	100+60µL	60+60µL

FaST program was created with higher dispense speeds for each step on the Digest Pro MSi robot in order to **reduce the processing time per sample from 40 min to 10min.**

Classic vs FaST desalting with 100ng of HeLa Digest (10ng injected)



A small increase (11% gain) in peptide intensities was observed with the FaST program (10min) compared to the classic program (40min). The **number of quantified proteins remains unchanged.**

Conclusion

- ✓ Better hydrophilic peptide retention
 - ✓ Increased peptide intensities
 - ✓ Higher number of proteins identified
- with **AttractSPE® Tips C18**

- ✓ Optimization of the desalting program on Digest Pro MSi robot with great time saving
- ✓ No cross-contamination observed with the robot

In collaboration with