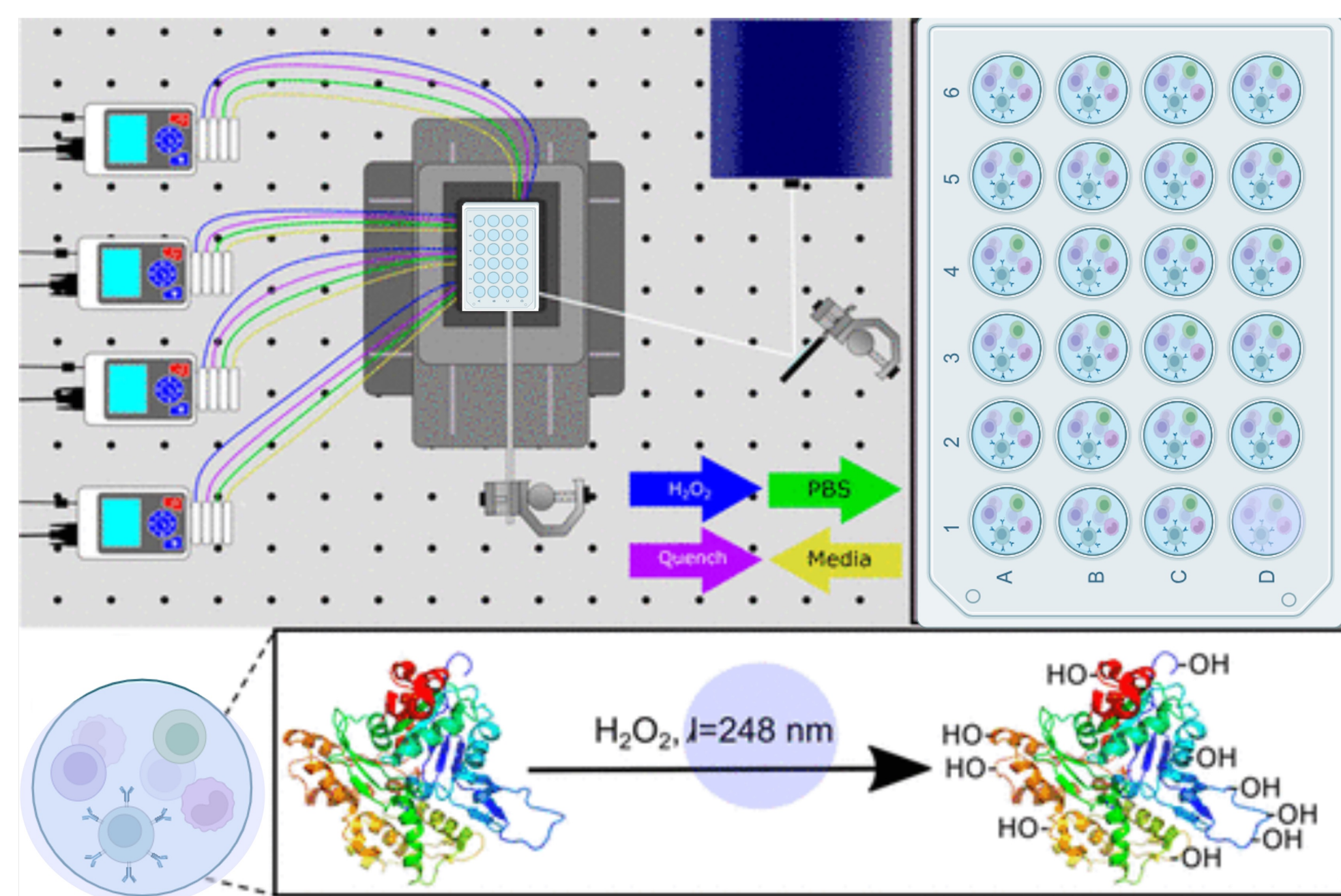


# Orbitrap Astral MS vs. Orbitrap Exploris 480 MS: Comparing the Identification of In-Cell Fast Photochemical Oxidation of Proteins Within HEK-293 and PBMCs

## 1. Background



In Cell Fast Photochemical Oxidation of Proteins (IC-FPOP) is a protein footprinting method that uses hydroxyl radicals to irreversibly label solvent accessible amino acid side chains. We developed a static, automated platform for performing IC-FPOP on adherent cells in 6-well plates called Platform Incubator with XY Movement (PIXY). The expansion of PIXY to labeling cells in 24-well plates would increase the throughput of IC-FPOP and provide the ability to study large patient sample cohorts. The addition of a cylindrical lens and adjustments to the angle of mirrors leads to the ability to irradiate an entire well in a 24-well plate in a single shot.

## 2. Instrumentation

A confluent well in a 24-well plate has less than half the number of cells of a 6-well plate. This would lead to low abundant samples. We compared 2 different MS instruments to determine the best for analyzing IC-FPOP in 24-well plates. **Advantages of Thermo Scientific™ Orbitrap Exploris™ 480 MS**

- High resolution up to 480,000
- Robust performance
- High throughput



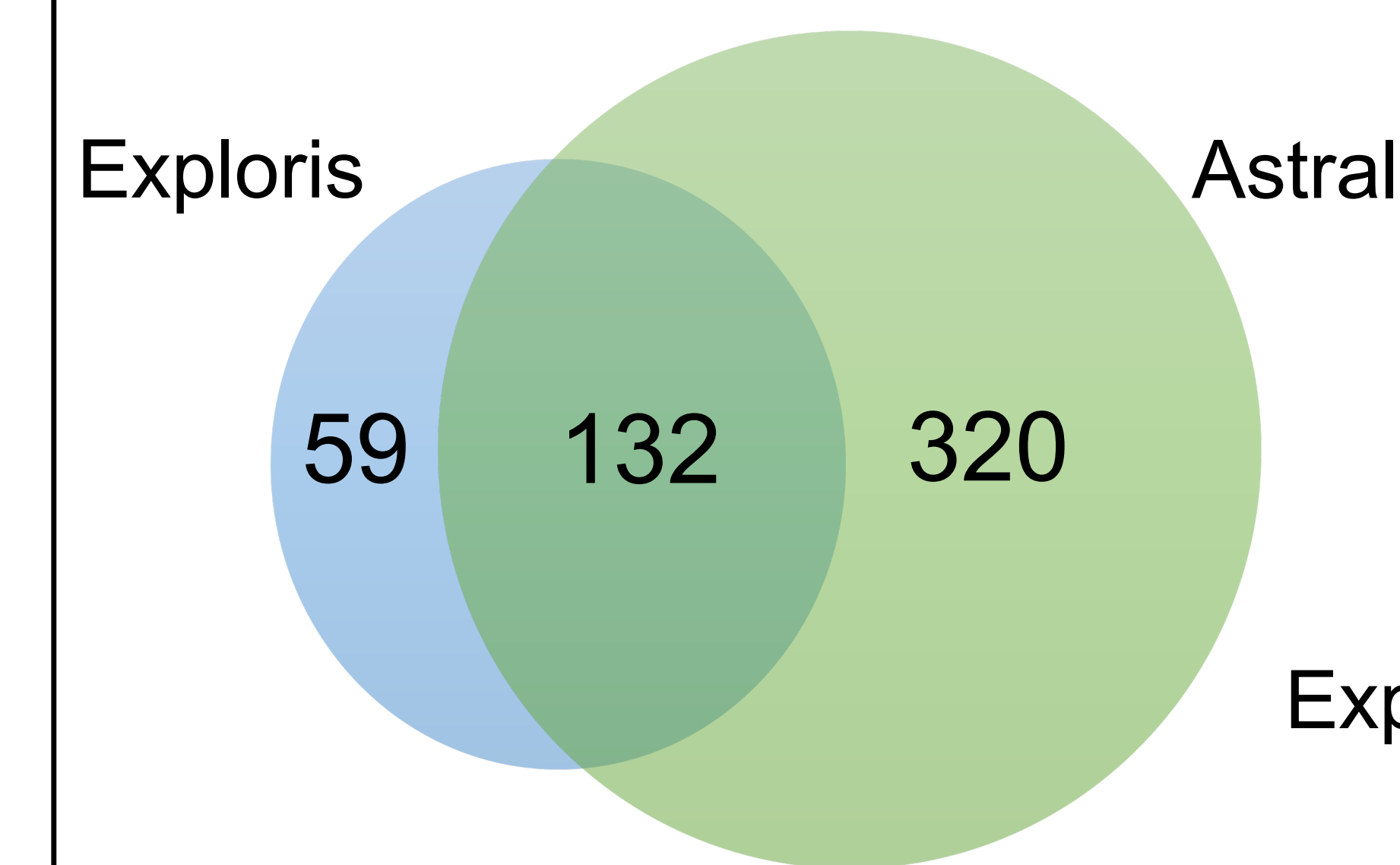
**Advantages of Thermo Scientific™ Orbitrap™ Astral™ MS**

- Faster throughput
- Deeper coverage
- Higher sensitivity
- Accurate and precise quantitation

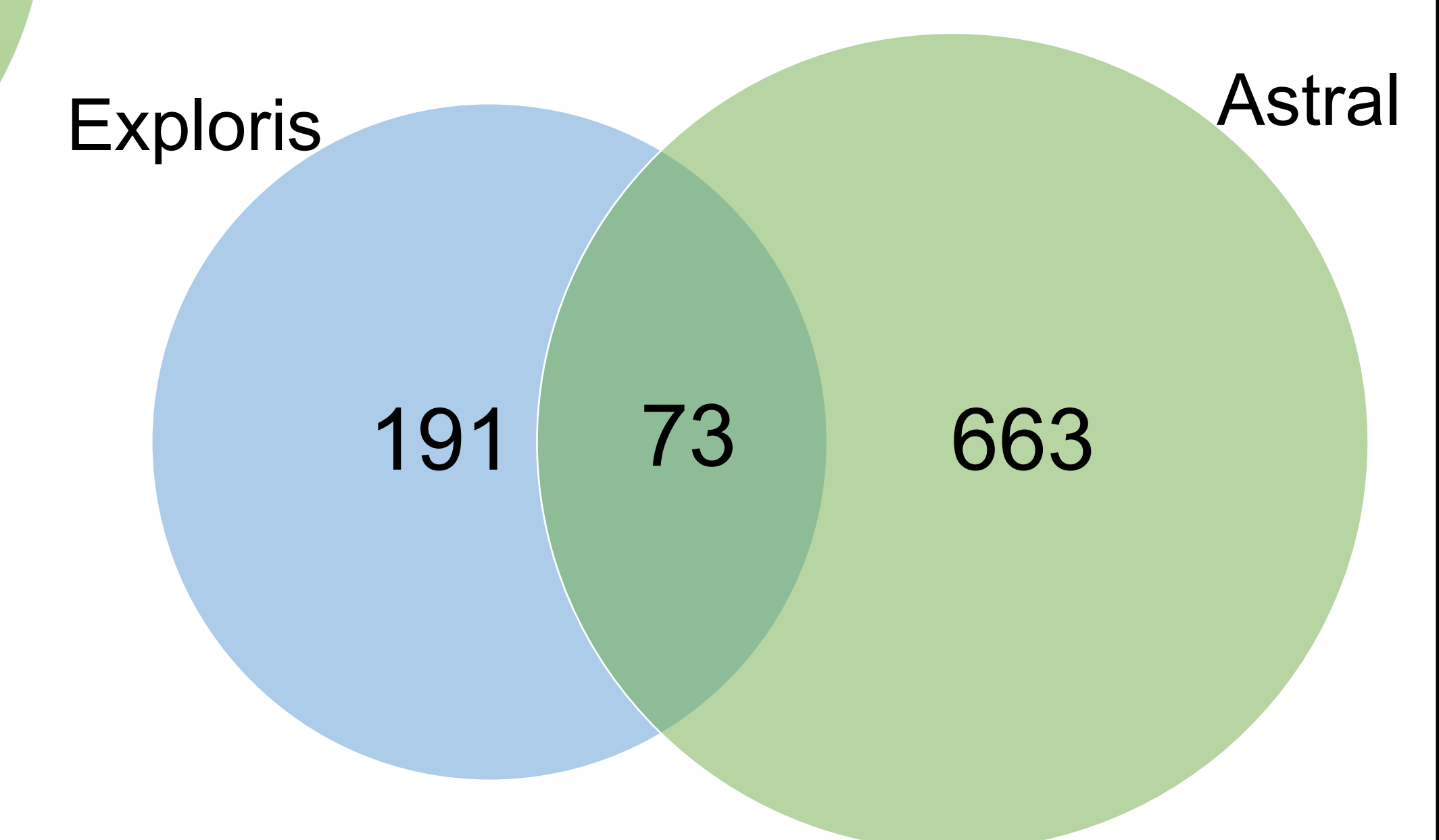


## 3. IC-FPOP Analysis on HEK-293 Cells

**Number of Quantifiable Modified Proteins**

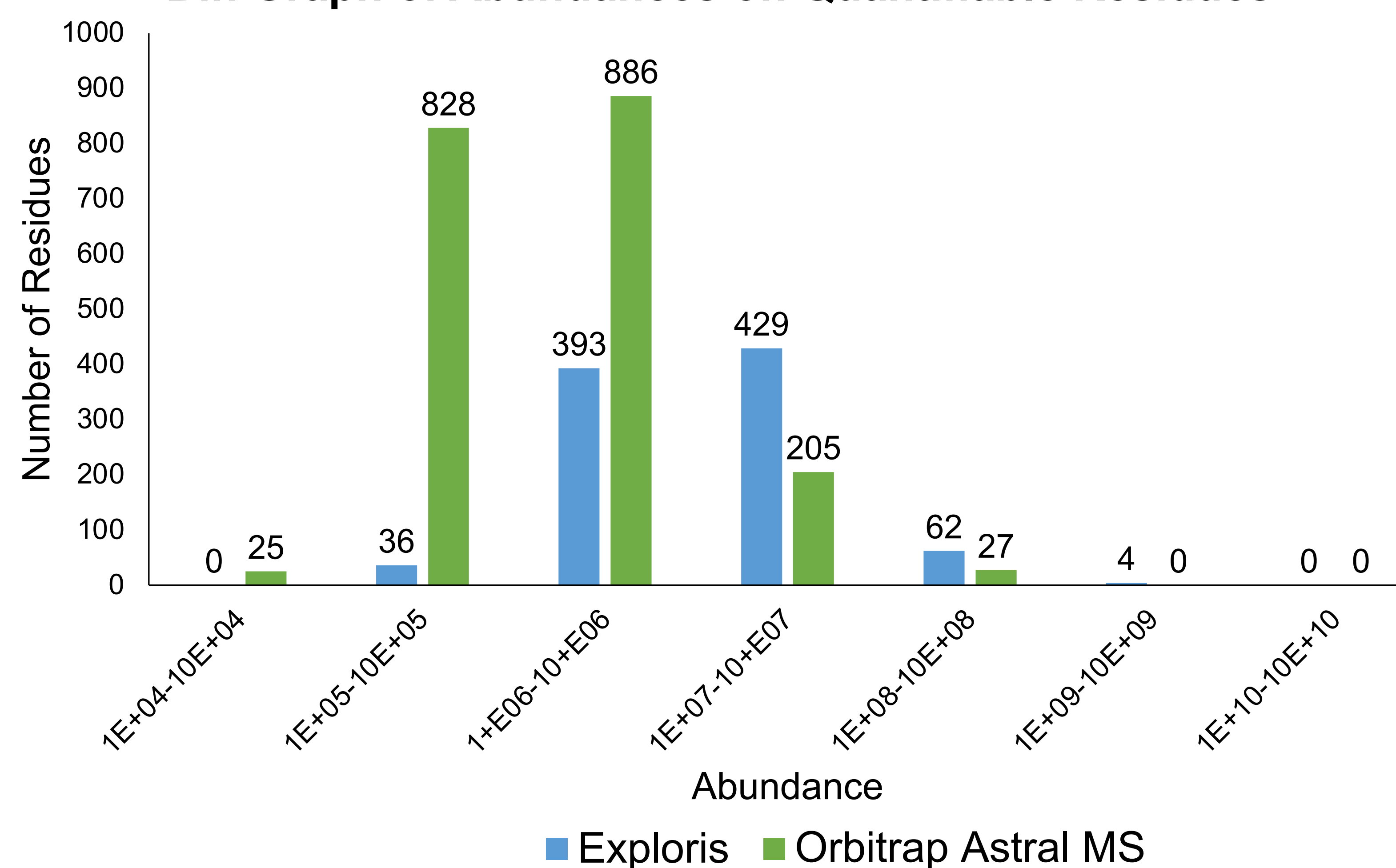


**Number of Quantifiable Modified Peptides**

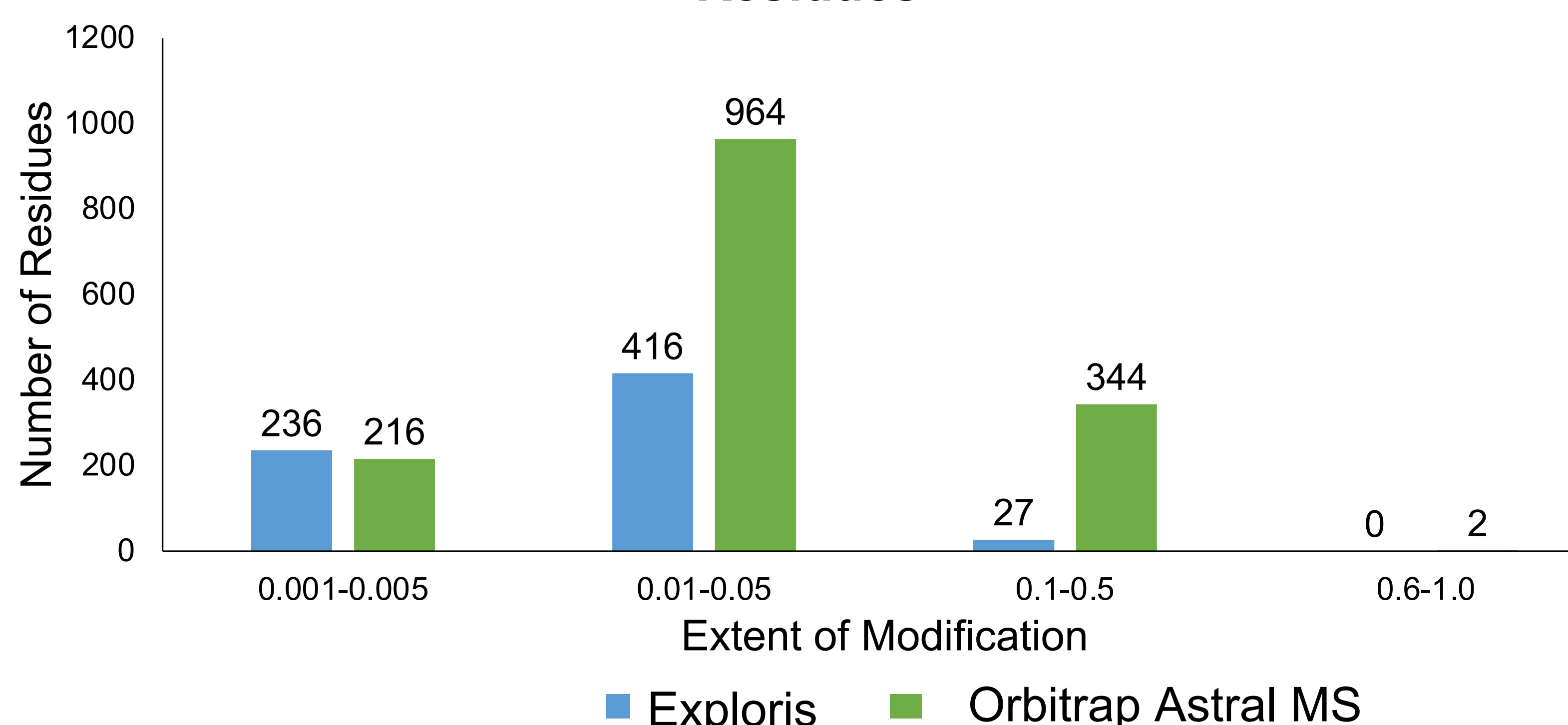


## 4. Analysis of Protein Abundance and EOM

**Bin Graph of Abundances on Quantifiable Residues**

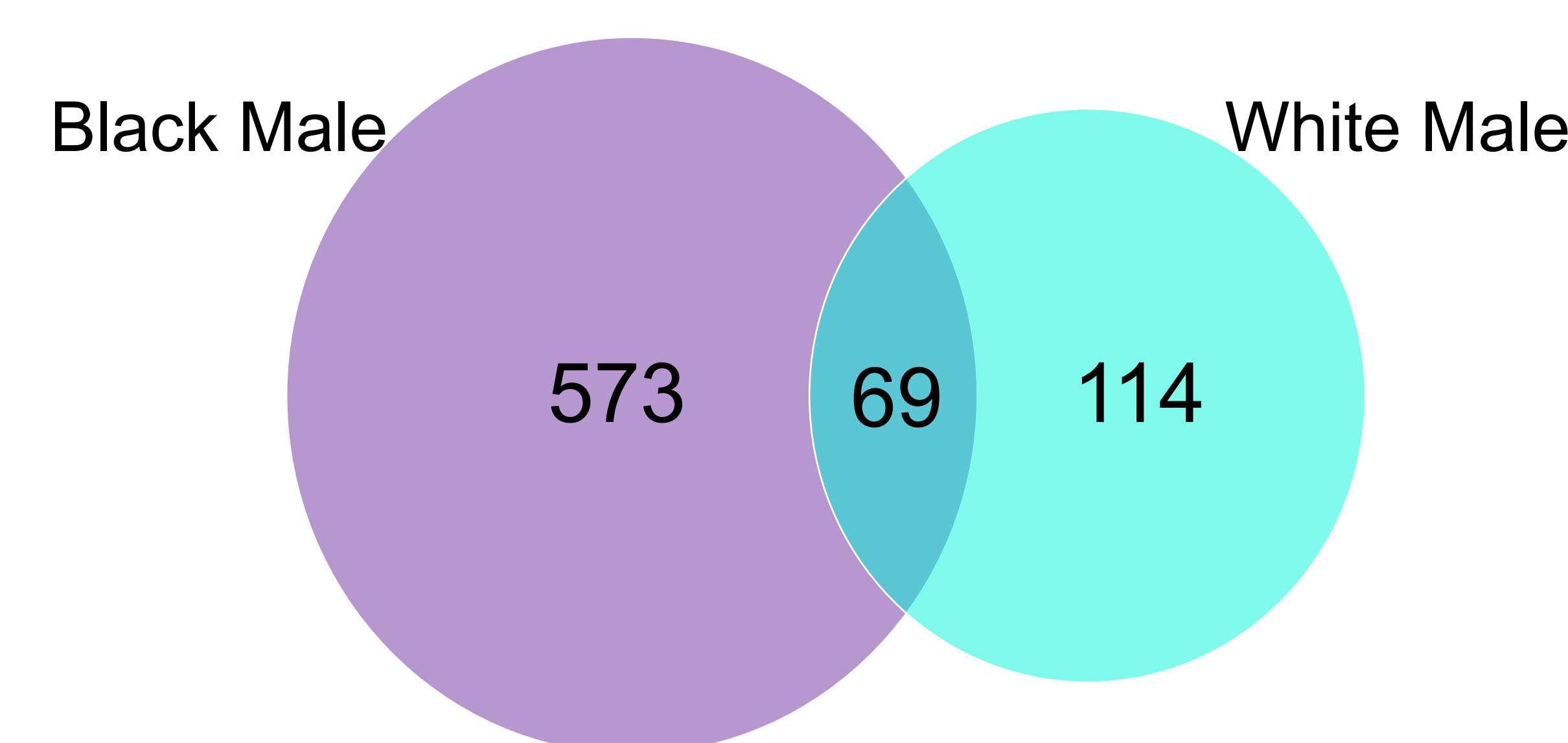


**Bin Graph of Extent of Modification on Quantifiable Residues**

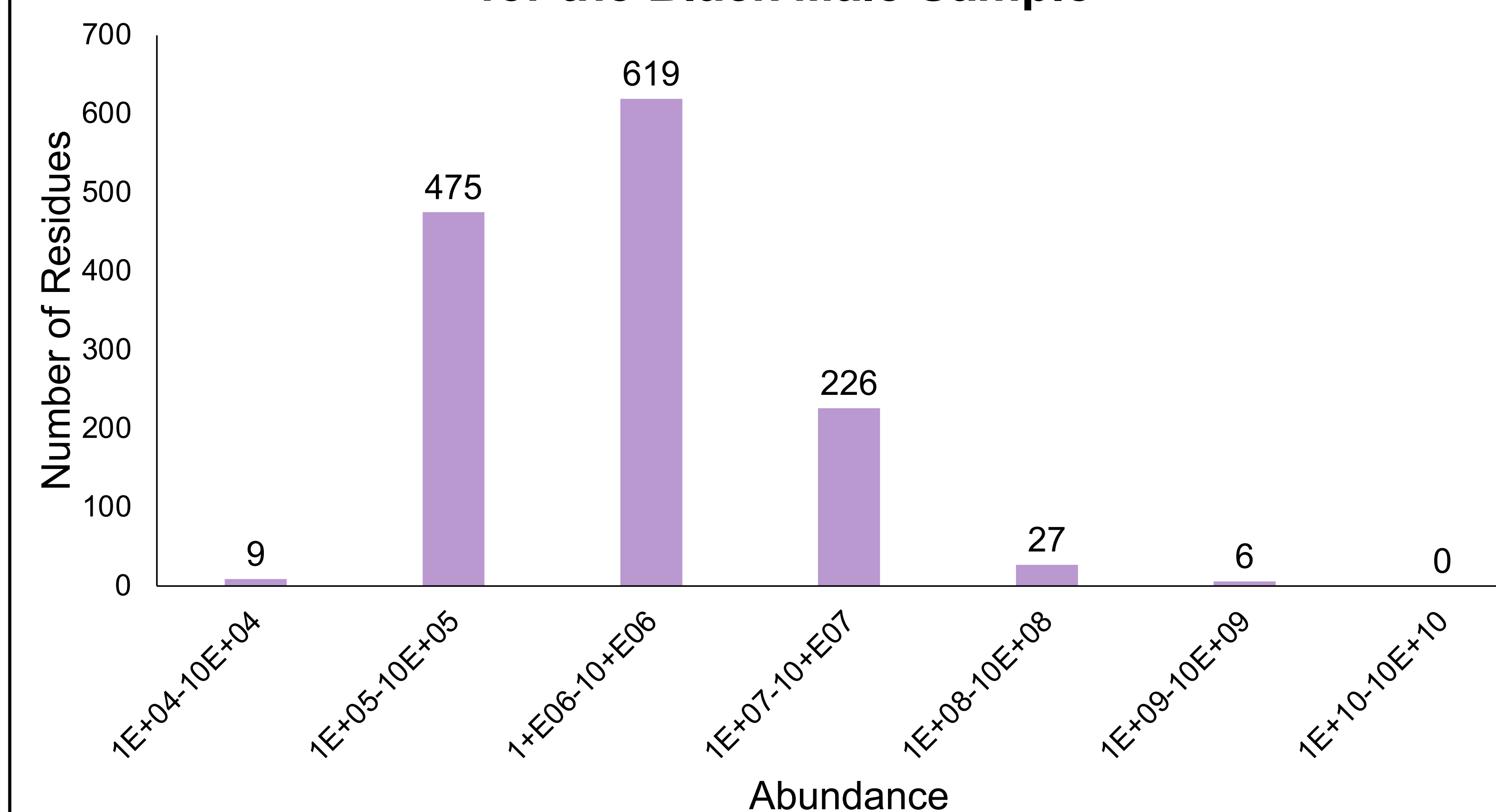


## 5. IC-FPOP Analysis on PBMCs

**Number of Quantifiable Modified Peptides**



**Bin Graph of Abundances on Quantifiable Residues for the Black Male Sample**



## 6. Conclusions

- IC-FPOP was adapted for 24-well plates to increase throughput.
- Orbitrap Astral MS showed a 1.3-fold increase in proteins identified and a 2.1-fold increase in peptide spectral matches compared to the Orbitrap Exploris 480 MS when searched on Thermo Scientific Proteome Discoverer software 3.1.
- The total number of modified residues as an average per protein was 50% for the Orbitrap Astral MS and 31% for the Orbitrap Exploris 480 MS.
- Orbitrap Astral MS identified more low abundance proteins than the Orbitrap Exploris 480 MS.
- IC-FPOP was performed on PBMCs from a black male and white male.
- The Orbitrap Astral MS identified 462 and 152 quantifiable modified proteins for the black male & white male respectively

## 7. Acknowledgements

1. Kaur, U. J. Am. Soc. Mass Spectrom. 2020.
2. Mass spectrometer images from Thermo Scientific. The authors acknowledge Dr. Raquel Shortt for the development of coADAPTr and her assistance in data analysis. This research was funded by the NIH R35GM144324. The authors declare no competing financial interest. PO003315