

# The role of oxidative post-translational modifications on the structure and activity of wound-related proteins

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## INTRODUCTION

What is cold physical plasma?

- Partially ionized gas, containing free electrons, reactive species and neutral particles (fourth state of matter)

Impact of cold physical plasma in chronic wounds:

- Accelerated healing [1]
- stimulation of tissue regeneration and modulation of inflammation, direct and indirect antimicrobial effects [2]
- modulation of biochemical signal pathways by short- and long-lived reactive oxygen and nitrogen species

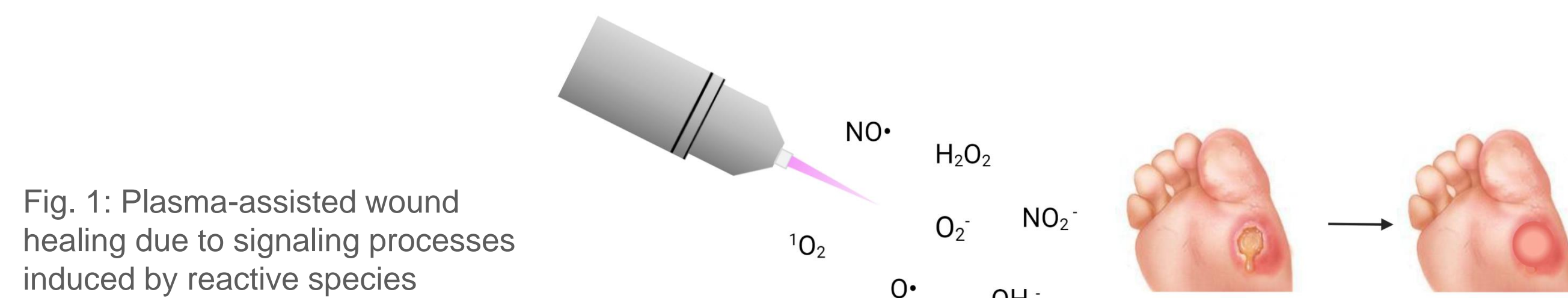


Fig. 1: Plasma-assisted wound healing due to signaling processes induced by reactive species

## SUMMARY & CONCLUSION

- The activity of wound-related enzymes is modulated by cold physical plasma in a treatment time depending manner
- The impact can be controlled by the discharge conditions – atomic and singlet oxygen show strongest impact (shielded Ar/O<sub>2</sub> conditions)
- Oxidation events in several amino acids observed (oxPTMs):
  - Catalase: heme channel and active site partially oxidised
  - Myeloperoxidase: tertiary structure formation compromised – cysteines involved in disulfide formation and binding site oxidised
- Changes in protein primary/secondary/tertiary structure observed
- Plasma treatment of chronic wounds potentially affects enzyme activity in the exsudate and modulates wound environment and healing

## ACTIVITY MEASUREMENT

Impact of treatment time and gas phase composition

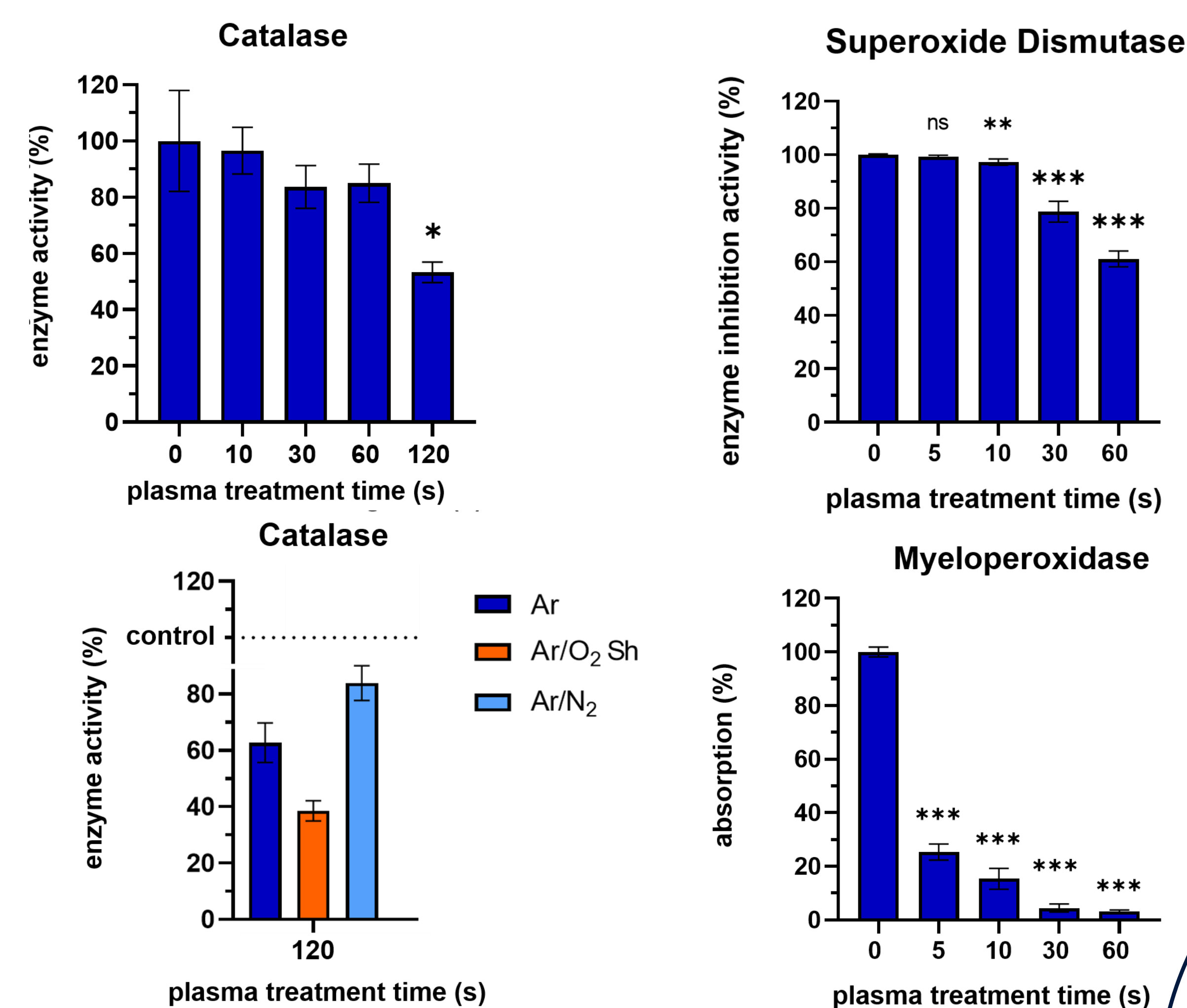


Fig. 2: The enzymatic activity of catalase, superoxide dismutase and myeloperoxidase decreases after plasma treatment in a time depending way. The greatest effect shows a composition of argon with 0.5 % oxygen and a nitrogen shielding gas.

## STRUCTURAL ANALYSIS

Impact on protein primary/secondary structure

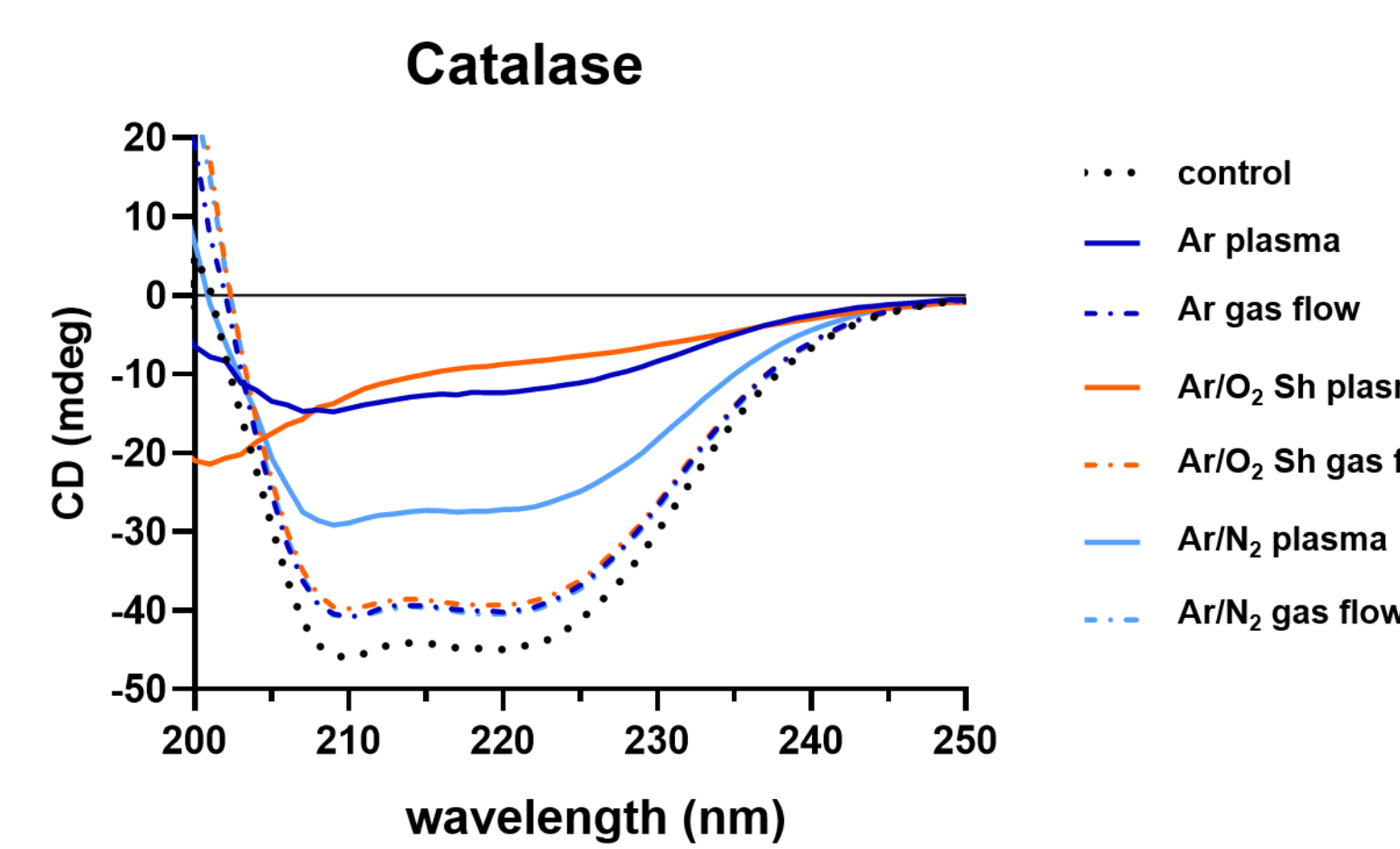


Fig. 3: CD-spectroscopy shows a conformational change of catalase after plasma treatment (loss of  $\alpha$ -helices).

Superoxide Dismutase

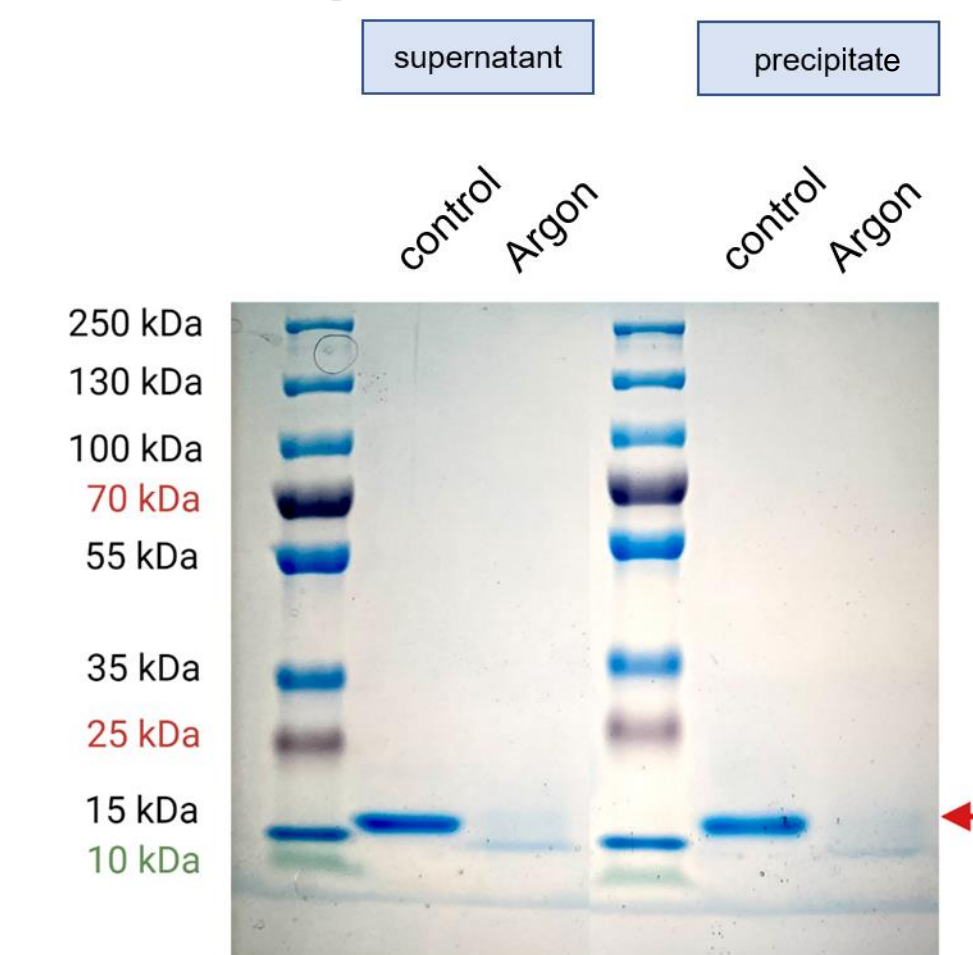


Fig. 4: SDS-Page of SOD indicates backbone cleavage or loss of protein.

Impact on protein size

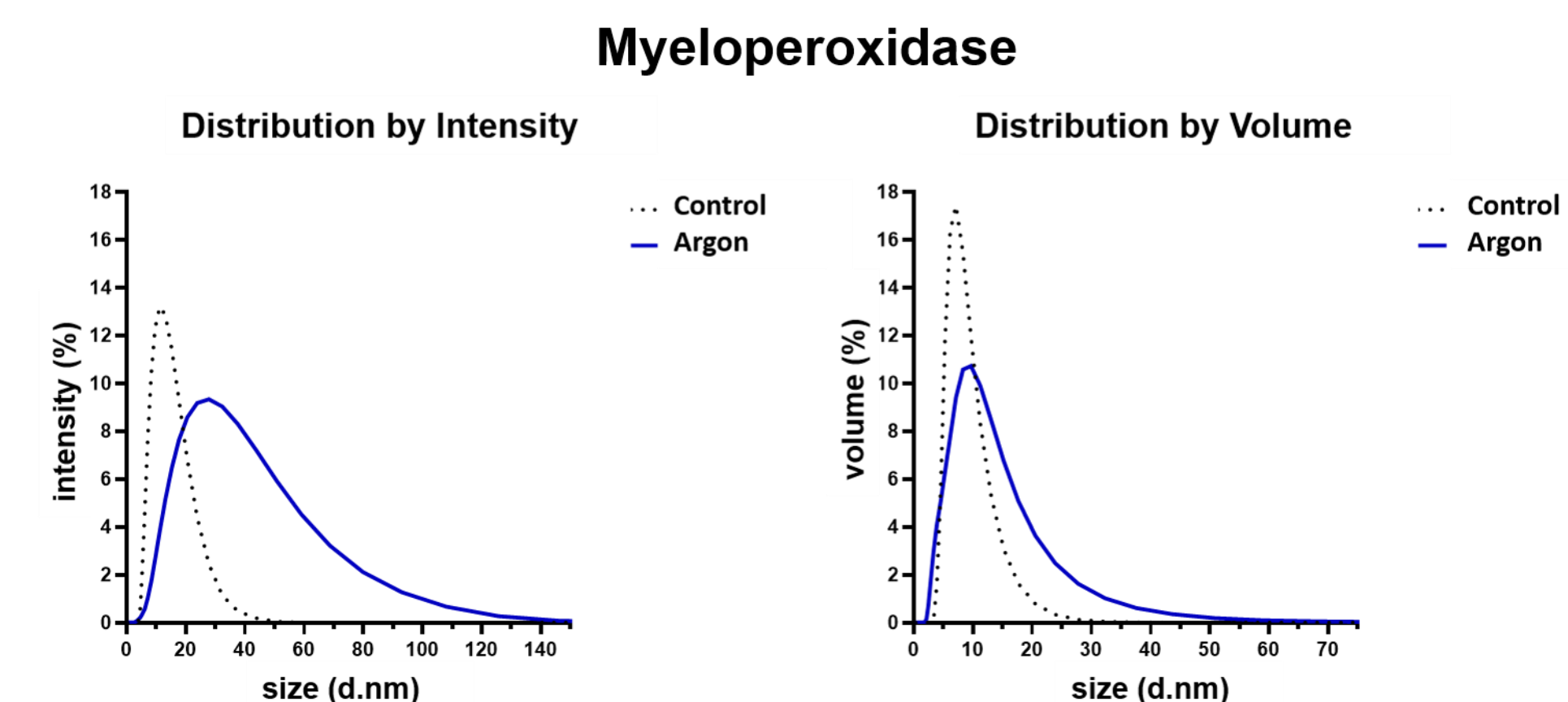


Fig. 5: Distribution by intensity and volume and correlation coefficient. The molecule size of MPO measured with DLS increases after plasma treatment.

## HIGH RESOLUTION MASS SPECTROMETRY:

Catalase

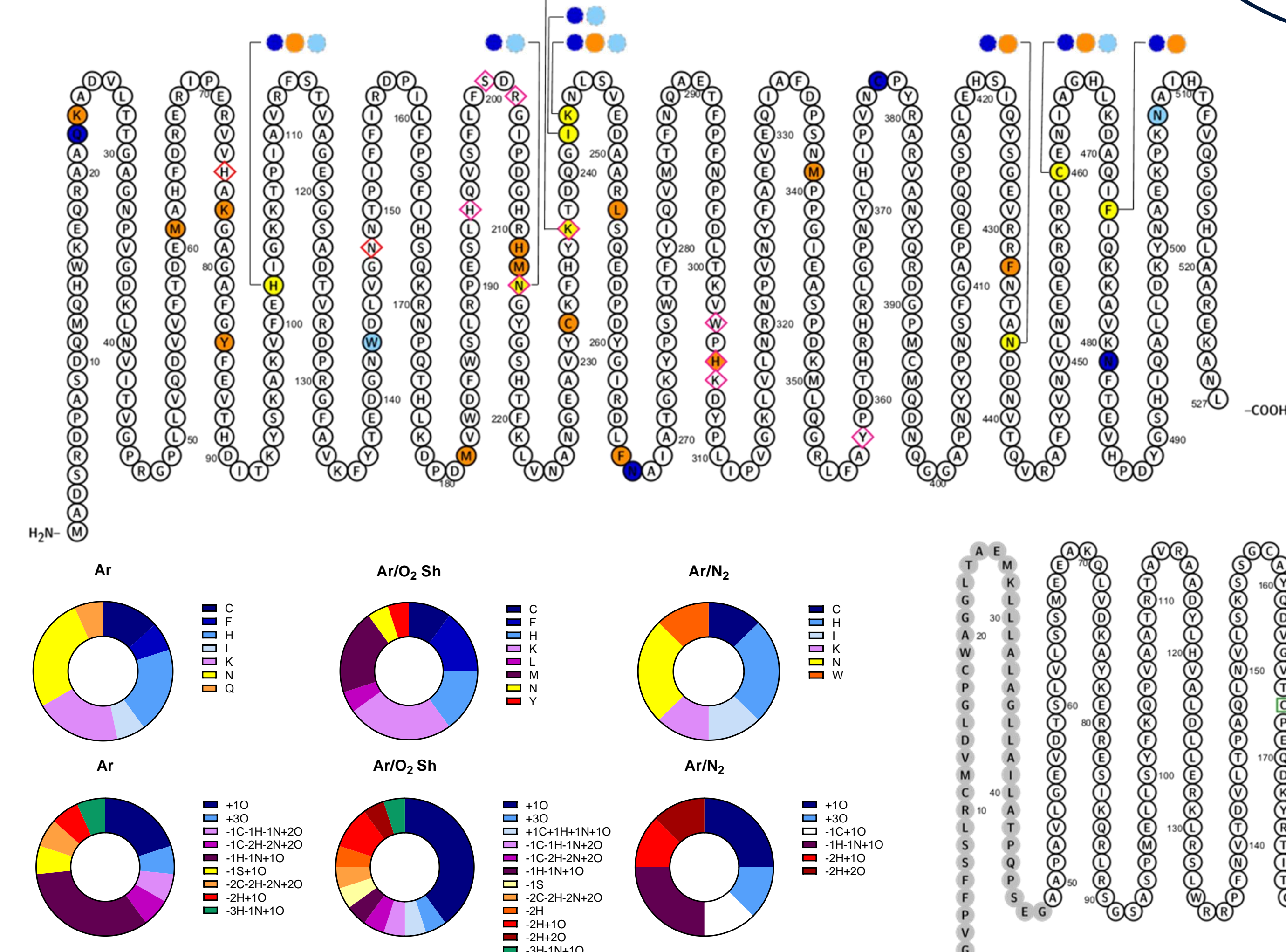


Fig. 6: Significant modifications of catalase after the plasma treatment (created with Protter) and the distribution of modified amino acids and types of modifications.

## OXIDATIVE POST-TRANSLATIONAL MODIFICATIONS

Myeloperoxidase

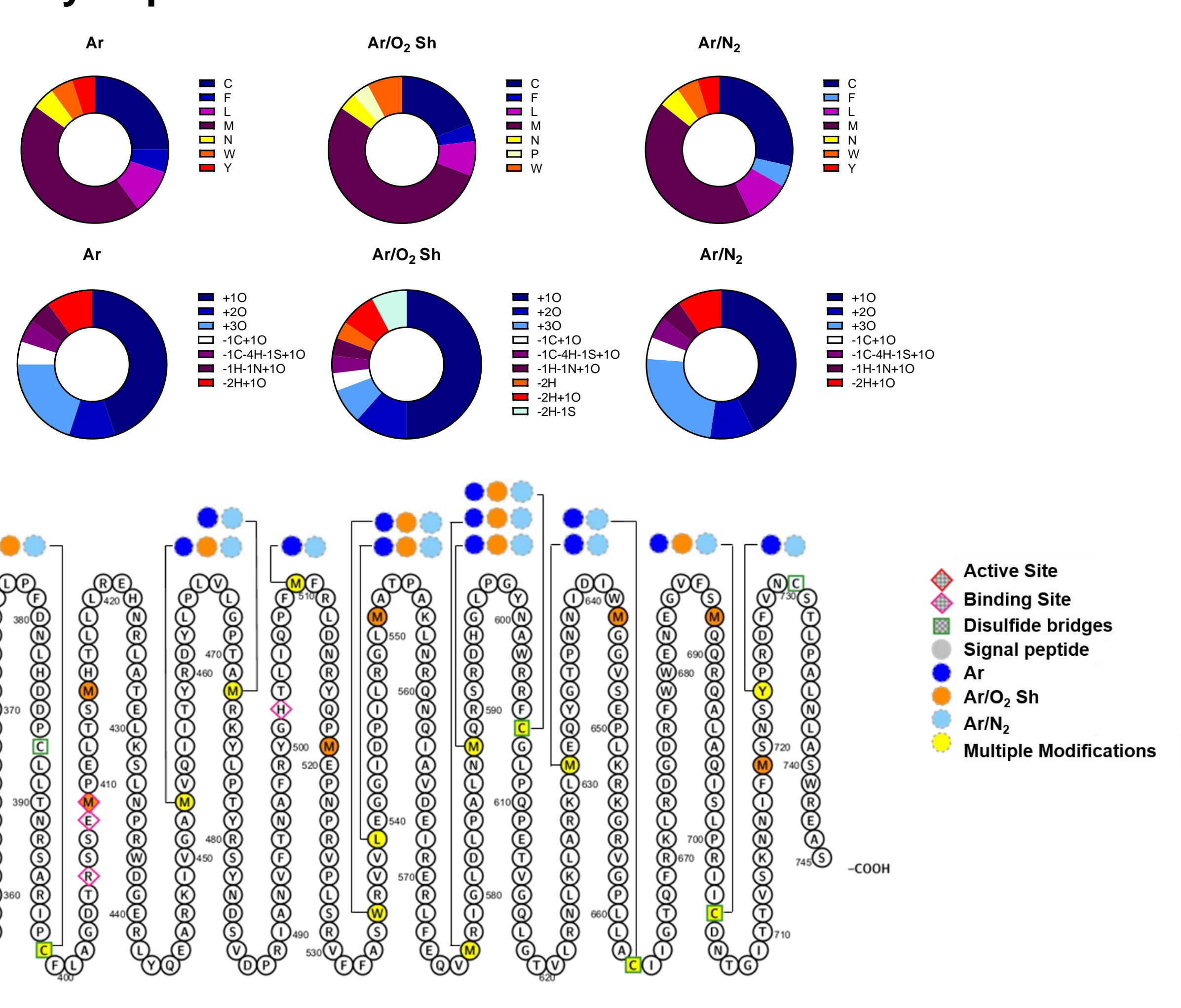


Fig. 7: Significant modifications of myeloperoxidase after the plasma treatment (created with Protter) and the distribution of modified amino acids and types of modifications.

### References:

- [1] B. Stratmann et al., "Effect of Cold Atmospheric Plasma Therapy vs Standard Therapy Placebo on Wound-Healing in Patients With Diabetic Foot Ulcers: A Randomized Clinical Trial," JAMA Netw Open, vol. 3, no. 7, p. e2010411, Jul 1 2020, doi: 10.1001/jamanetworkopen.2020.10411
- [2] A. Schmidt, G. Liebert, F. Niessner, T. von Woedtke, and S. Bekeschus, "Gas plasma-spurred wound healing is accompanied by regulation of focal adhesion, matrix remodelling, and tissue oxygenation," Redox Biol, vol. 38, p. 101809, Jan 2021, doi: 10.1016/j.redox.2020.101809