The First Pilot Project of the Consortium for Top-Down Proteomics: A Status Report

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Summary

Pilot Project #1 - the identification and characterization of human histone H4 proteoforms by top-down MS - is the first project launched by the Consortium for Top-Down Proteomics (CTDP) to refine and validate top-down MS/MS. Among the initial results from seven participating laboratories, all reported the probability-based identification of human histone H4 (UniProt accession P62805) with expectation values ranging from 10^{-13} to 10^{-105}. Regarding characterization, 74 total proteoforms were reported, with 21 done so unambiguously; one new post-translational modification (PTM), K79ac, was identified. Inter-laboratory comparison (Fig. 1) reveals some consistent results, such as the localization of individual PTMs and binary combinations, whereas other aspects are more variable, such as the accurate characterization of low-abundance proteoforms harboring more than two PTMs. An open-access tool and discussion of proteoform scoring are included, along with a description of general challenges that lie ahead, including improved proteoform separations prior to mass spectrometric analysis, better instrumentation performance, and software development.

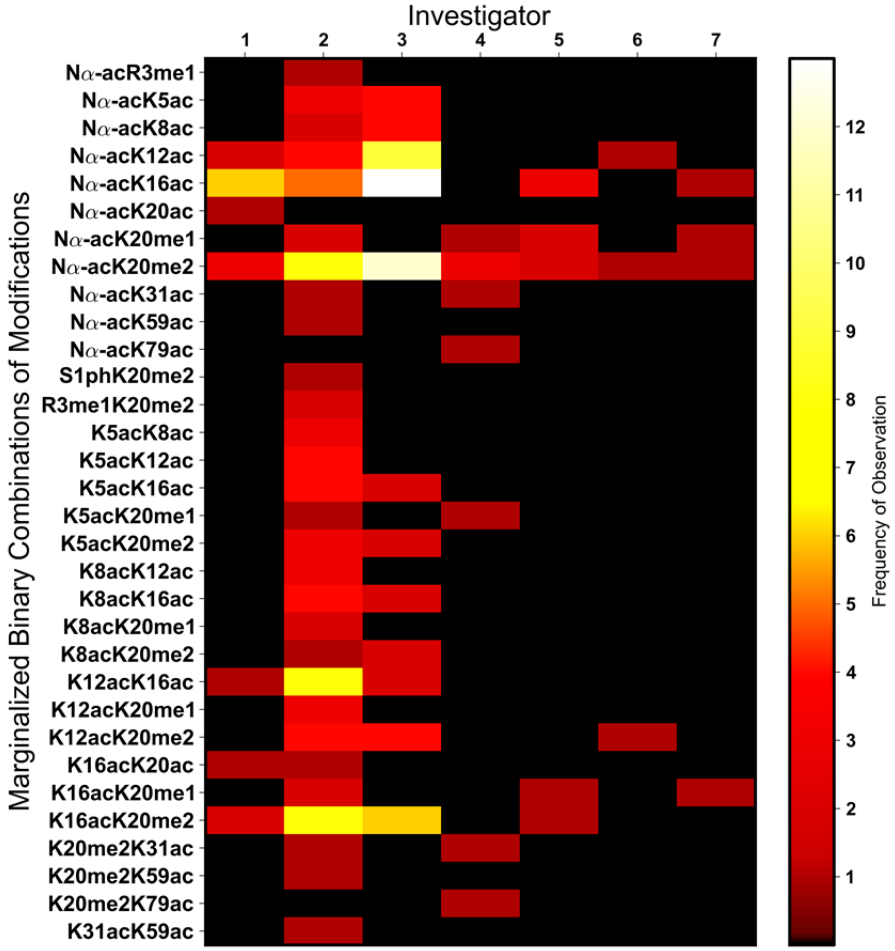

Fig. 1 Heat map shwoing the binary combinations of PTMs marginalized from the collective top-down data, organized by investigator. This marginalization is achieved by summing the number of observations of proteoforms that contain both PTMs. Only unambiguously localized PTMs are considered.

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References

[1] Dang, X., *et al*., Proteomics, **14**, 1130-1140 (2014).