

# Impact of sample preparation methods on proteoform identification by top-down proteomics

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Numerous workflows have been developed for top-down proteomics (TDP). We systematically investigated the influence of different sample preparation steps on proteoform and protein identifications, including cell lysis, reduction and alkylation, proteoform enrichment, purification, and fractionation [1]. We found that all steps in sample preparation influence the subset of proteoforms identified (e.g., their number, confidence, physicochemical properties, and artificially generated modifications). The various sample preparation strategies resulted in complementary identifications, significantly increasing the proteome coverage. Overall, more than 13,000 proteoforms from more than 2,700 proteins of human Caco-2 cells were identified.

The results presented can serve as suggestions for designing and adapting TDP sample preparation strategies to particular research questions. Moreover, the sampling bias and modifications identified at the intact protein level will also be useful in improving bottom-up proteomics approaches.

[1] Kaulich PT, Jeong K, Kohlbacher O, Tholey A (2024). Influence of different sample preparation approaches on proteoform identification by top-down proteomics. Nat Methods, 21: 2397-2407. doi: 10.1038/s41592-024-02481-6.

## User consent

yes

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