Lysis buffer selection guidance for mass spectrometry-based global proteomics including studies on the intersection of signal transduction and metabolism (preprint)

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Abstract

Prerequisite for a successful proteomics experiment is a high-quality lysis of the sample of interest, resulting in a large number of identified proteins as well as a high coverage of protein sequences. Therefore, the choice of suitable lysis conditions is crucial. Many buffers were previously employed in proteomics studies, yet a comprehensive comparison of lysate preparation conditions was so far missing. In this study, we compared the efficiency of four commonly used lysis buffers, containing the agents NP40, SDS, urea or GdnHCl, in four different types of biological samples (suspension and adherent cell lines, primary mouse cells and mouse liver tissue). After liquid chromatography-mass spectrometry (LC-MS) measurement and MaxQuant analysis, we compared chromatograms, intensities, number of identified proteins and the localization of the identified proteins. Overall, SDS emerged as the most reliable reagent, ensuring stable performance and reproducibility across diverse samples. Furthermore, our data advocated for a dual-sample lysis approach, including that the resulting pellet is lysed again after the initial lysis with a urea lysis buffer and subsequently both lysates are combined for a single LC-MS run to maximize the proteome coverage. However, none of the investigated lysis buffers proved to be superior in every category, indicating that the lysis buffer of choice depends on the proteins of interest and on the biological question. Further, we demonstrated with our systematic studies the establishment of conditions that allows to perform global proteomics and affinity purification-based interactome characterization from the same lysate. In sum our results provide guidance for the best-suited lysis buffer for mass spectrometry-based proteomics depending on the question of interest.