



Ultra-high sensitivity mass spectrometry quantifies single-cell proteome changes upon perturbation

Andreas-David Brunner¹ , Marvin Thielert¹, Catherine Vasilopoulou¹, Constantin Ammar¹ , Fabian Coscia² , Andreas Mund² , Ole B Hoerning³, Nicolai Bache³, Amalia Apalategui⁴, Markus Lubeck⁴, Sabrina Richter^{5,6} , David S Fischer^{5,6} , Oliver Ræther⁴, Melvin A Park⁷, Florian Meier^{1,8} , Fabian J Theis^{5,6}  & Matthias Mann^{1,2,*} 

Abstract

Single-cell technologies are revolutionizing biology but are today mainly limited to imaging and deep sequencing. However, proteins are the main drivers of cellular function and in-depth characterization of individual cells by mass spectrometry (MS)-based proteomics would thus be highly valuable and complementary. Here, we develop a robust workflow combining miniaturized sample preparation, very low flow-rate chromatography, and a novel trapped ion mobility mass spectrometer, resulting in a more than 10-fold improved sensitivity. We precisely and robustly quantify proteomes and their changes in single, FACS-isolated cells. Arresting cells at defined stages of the cell cycle by drug treatment retrieves expected key regulators. Furthermore, it highlights potential novel ones and allows cell phase prediction. Comparing the variability in more than 430 single-cell proteomes to transcriptome data revealed a stable-core proteome despite perturbation, while the transcriptome appears stochastic. Our technology can readily be applied to ultra-high sensitivity analyses of tissue material, posttranslational modifications, and small molecule studies from small cell counts to gain unprecedented insights into cellular heterogeneity in health and disease.

Keywords drug perturbation, low-flow LC-MS, proteomics at single-cell resolution, single-cell heterogeneity, systems biology

Subject Categories Cell Cycle, Proteomics

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Introduction

In single-cell analysis, biological variability can directly be attributed to individual cells instead of being averaged over an ensemble or complex tissue (Regev *et al.*, 2017). While microscopy has always been single-cell based, specialized deep sequencing technologies have achieved this for systems biological approaches (Smith *et al.*, 2010; Ramsköld *et al.*, 2012; Jaitin *et al.*, 2014; Schmitzbaumer *et al.*, 2017; Schaum *et al.*, 2018; Lundberg & Börner, 2019). At the level of proteins, the functional actors of cells, single cells are currently studied by antibody-based technologies, which are by necessity directed against previously chosen targets (Uhlén *et al.*, 2015; Söeckiüs *et al.*, 2017; Jackson *et al.*, 2020). In contrast, mass spectrometry (MS)-based proteomics is unbiased in the sense that it measures all proteins within its range of detection (Larance & Lamond, 2015; Aebersold & Mann, 2016). Thus, it would be highly desirable to apply this technology to single cells if the required sensitivity and robustness could be achieved. Previous approaches that employed chemical multiplexing of peptides have labeled a small number of single cells but combined them with a dominant booster channel for MS analysis (Budnik *et al.*, 2018; Tsai *et al.*, 2020; Schoof *et al.*, 2021), which can hamper signal deconvolution (Bremer *et al.*, 2019; Cheung *et al.*, 2021). Alternatively, proof of principle has been demonstrated for unlabeled approaches using sophisticated sample preparation methods in pico-liter devices (Li *et al.*, 2018; Liang *et al.*, 2020; Williams *et al.*, 2020).

Here, we set out to develop an ultrasensitive MS-based workflow that would allow quantitatively precise and accurate MS proteomics data by injecting single cells one by one into the MS—which we call true single-cell-derived proteomics (T-SCP). To achieve scale, robustness, and community adoption, we aimed to combine technologies that could readily be made commercially available. We

1 Proteomics and Signal Transduction, Max-Planck Institute of Biochemistry, Martinsried, Germany

2 NMF Center for Protein Research, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark

3 EvoSep Biosystems, Odense, Denmark

4 Bruker Daltonik GmbH, Bremen, Germany

5 Helmholtz Zentrum München - German Research Center for Environmental Health, Institute of Computational Biology, Neuherberg, Germany

6 TUM School of Life Sciences Weihenstephan, Technical University of Munich, Freising, Germany

7 Bruker Daltonics Inc., Billerica, MA, USA

8 Functional Proteomics, Jena University Hospital, Jena, Germany

*Corresponding author. E-mail: mmann@biochem.mpg.de

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