PCP
PLANT & CELL PHYSIOLOGY

Advances in single-cell proteomics: label-free quantification of over 3500 protein groups from individual *Arabidopsis thaliana* seedling cells

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Received 24 July 2026; Revised 30 September 2026; Accepted 30 September 2026

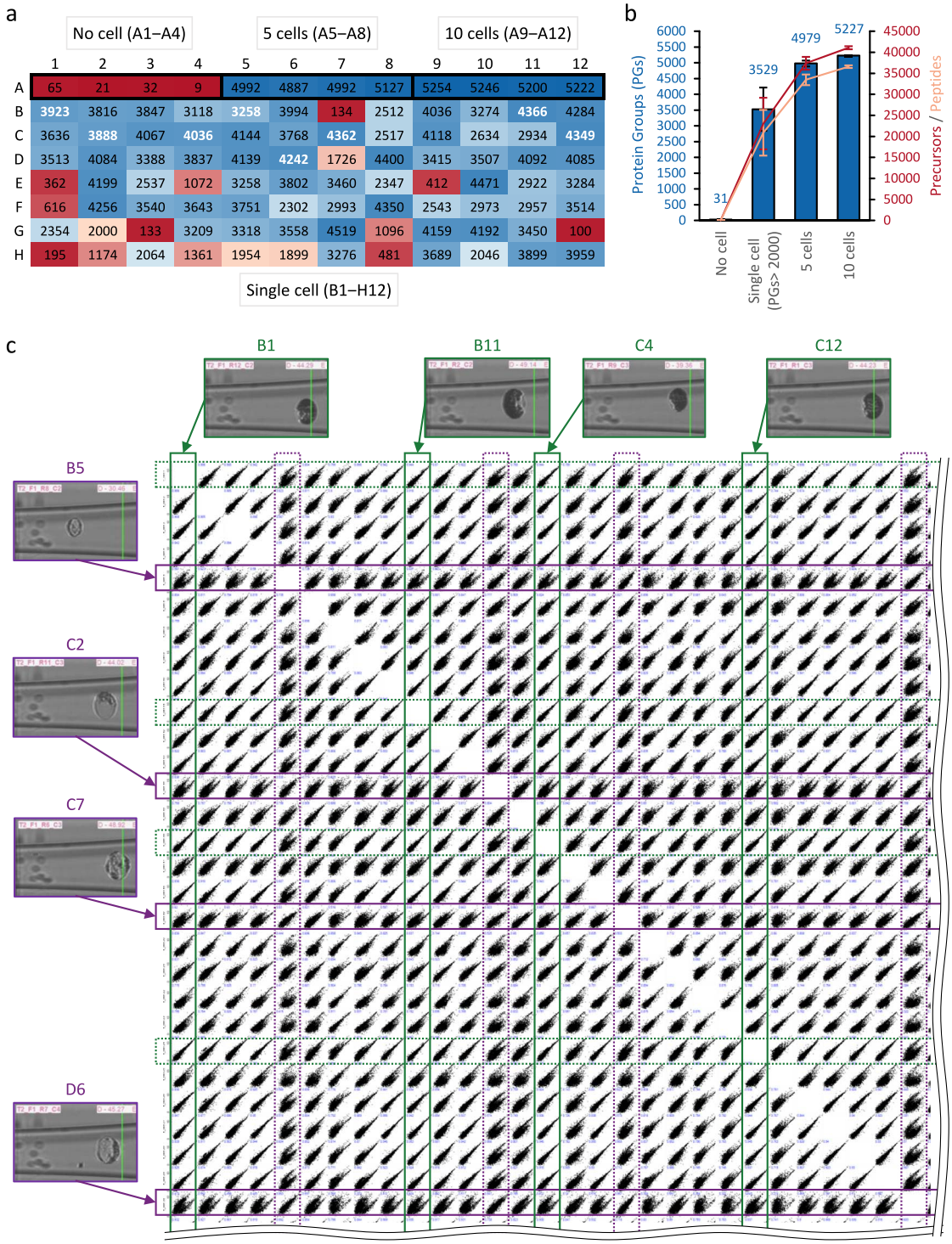
Recent single-cell omics studies in plants, primarily focused on gene expression and its regulatory landscape, have underscored the importance of monitoring cell-type-specific responses, as well as cellular heterogeneity and distinct cell states within the same cell type (Nobori 2025). Protein abundance and activity are not always reflected by transcript levels, highlighting the potential of single-cell proteomics to provide complementary insights into plant biological systems (Walley et al. 2016, Mergner et al. 2020, Clark et al. 2022, Fulcher et al. 2026). A major challenge in single-cell proteomics is minimizing sample loss during preparation while maximizing the detection of low-abundance peptides by liquid chromatography–mass spectrometry (LC–MS), as protein signals cannot be amplified in the same way as genomic or transcriptomic material. Nevertheless, recent advances in sample preparation, LC–MS instrumentation, and data analysis have enabled the identification and quantification of thousands of proteins from individual mammalian cells (Bubis et al. 2025, Ye et al. 2025, Brenes et al. 2026, Slavov 2026). In plants, however, single-cell proteomics remains in its infancy. To date, only two pioneering studies have reported single-cell proteomics in plants, demonstrating its ability to distinguish both cell types and cell states (Montes et al. 2024, Fulcher et al. 2026).

Montes *et al*. employed fluorescence-activated cell sorting of individual *Arabidopsis thaliana* root protoplasts to isolate cortex and endodermis cells, which were subsequently analysed by data-dependent acquisition (DDA) coupled with tandem mass tag labelling (Montes et al. 2024). From 756 root cells, the authors identified a total of 3763 proteins, with an average of 1118 proteins identified per cell, enabling the discrimination of cortex and endodermis cells based on their proteomic profiles. Fulcher *et al*. isolated individual *A. thaliana* leaf mesophyll protoplasts using the cellenONE platform and analysed them using a label-free DDA-based approach (Fulcher et al. 2026). The authors identified an average of 3003 proteins from single mesophyll protoplasts and 3278 proteins from pools of 10 protoplasts, serving as an initial performance assessment of the system prior to biological application. Across mesophyll cells subjected to moderate and severe water-deficit stress and corresponding controls, 117 protoplasts were initially analysed, with quality control filtering yielding 87 high-quality single cells based on visual inspection enabled by the cellenONE platform. From these, an average of 2873 proteins were identified per cell, representing a total of 3587 proteins identified across all cells. Downstream analysis of the single-cell proteomes resolved 18 protein clusters, revealing distinct proteomic states across the mesophyll cell population.

Here, we assessed the performance of a more recent data-independent acquisition (DIA) approach using updated LC–MS instrumentation (Supplementary Data) (Brenes et al. 2026). Protoplasts with a diameter of 30–50 μm and a maximum elongation of 1.7 were sorted into a 96-well plate using the cellenONE platform (Supplementary Figs. S1 and S2). Four wells were allocated for buffer-only controls, and four wells each for pooled samples (5-cell and 10-cell), while single cells were allocated to the remaining 84 wells (Fig. 1a). Sample preparation, including protoplast lysis and protein digestion, was performed directly in the 96-well plate following cell sorting using the cellenONE platform (Supplementary Data). The samples were subsequently analysed on a timsUltra Athena Ion Processor (AIP) operated in dia-Parallel Accumulation Serial Fragmentation (PASEF) mode (Supplementary Data). To assess the protein content per protoplast, we extracted protein from aliquots of 40 000 cells and determined the protein concentration, yielding an average of 2.44 ng per cell (three measurements, Supplementary Data). MS data were processed using Spectronaut software (Supplementary Data). Approximately 5000 protein groups were identified and quantified from the pooled samples, with strong correlations among all eight samples, suggesting protein identification saturation around the 5-cell level under the applied LC–MS settings and demonstrating the robustness of the approach

Plant and Cell Physiology, 2026, 00(00),1–4. https://doi.org/10.1093/pcp/pcag138
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	1	2	3	4	5	6	7	8	9	10	11	12
A	65	21	32	9	4992	4887	4992	5127	5254	5246	5200	5222
B	3923	3816	3847	3118	3258	3994	134	2512	4036	3274	4366	4284
C	3636	3888	4067	4036	4144	3768	4362	2517	4118	2634	2934	4349
D	3513	4084	3388	3837	4139	4242	1726	4400	3415	3507	4092	4085
E	362	4199	2537	1072	3258	3802	3460	2347	412	4471	2922	3284
F	616	4256	3540	3643	3751	2302	2993	4350	2543	2973	2957	3514
G	2354	2000	133	3209	3318	3558	4519	1096	4159	4192	3450	100
H	195	1174	2064	1361	1954	1899	3276	481	3689	2046	3899	3959

Figure 1. Protein identification from sorted *A. thaliana* protoplasts. (a) Number of protein groups identified from each well of a 96-well plate into which protoplasts were sorted. Wells A1–A4 contained no cells (0-cell controls), A5–A8 contained 5 cells each, A9–A12 contained 10 cells each, and wells B1–H12 each contained a single cell. MS data were processed using the Spectronaut classical DIA workflow. The spectral library was generated from the 5- and 10-cell samples. Protein identification numbers for the cells selected in (c) are highlighted in white. (b) Number of protein groups, precursors, and peptides identified from 0-, 5-, 10-, and single-cell samples using the Spectronaut classical DIA workflow. Only single-cell samples with more than 2000 identified protein groups were included in the analysis. The spectral library was generated from the 5- and 10-cell samples. Error bars represent the standard deviation of the mean. (c) Multiscatter plot of log2 transformed LFQ intensities of identified proteins across the selected single-cell samples. Four cells (B5, C2, C7, and D6: highlighted with purple boxes) showed weak correlation with the remaining cells. Images of eight selected sorted protoplasts are shown. The four cells exhibiting poor correlation most likely lacked chloroplasts. Images of all sorted protoplasts are provided in Supplementary Fig. S2. The complete multiscatter plot is provided in Supplementary Fig. S7.

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(Fig. 1a, Supplementary Fig. S3). From single cells, an average of 3061 protein groups per cell was identified across all wells (Supplementary Fig. S4). In some wells, substantially lower numbers of proteins were detected, likely due to damaged protoplasts or errors during sample preparation (Fig. 1a, Supplementary Fig. S2). Therefore, 68 wells with more than 2000 identified protein groups were selected for further analyses (Fig. 1a). From the selected wells, an average of 3529 protein groups per cell was identified, resulting in a dataset comprising 5323 unique protein groups (Fig. 1b). Compared with the 3587 proteins identified by Fulcher *et al.*, more than 2000 proteins were uniquely identified in our dataset (Supplementary Fig. S5) (Fulcher et al. 2026). Importantly, substantially more transcription factors and protein kinases were uniquely identified in this study, which may provide improved coverage for network analyses aimed at investigating cellular states (Supplementary Fig. S5). Overall, the detected proteomes of the selected cells showed strong correlations (Fig. 1c). Intriguingly, however, the proteomes of four cells showed weak correlations with the rest of the dataset (Fig. 1c). Visual inspection of these isolated protoplasts revealed that these cells lacked chloroplasts, suggesting that they may represent different cell types or residual root-derived material that was not fully excluded during protoplast preparation (Fig. 1c, Supplementary Fig. S2). This observation suggests that the label-free DIA-based approach applied here can resolve cellular heterogeneity without prior annotation in plants. Further, more sophisticated analyses of the remaining cells, such as clustering, may reveal additional cell types or states. The MS data are available via ProteomeXchange with identifier PXD080545.

In summary, we demonstrate that single-cell proteomics technologies are maturing to a level at which more than 4400 protein groups can be identified and quantified from single plant cells, enabling the detection of cellular heterogeneity (Fig. 1a and c). Of note, directDIA data processing of single-cell data without the use of a spectral library identified nearly 4500 protein groups across all cells and close to 4000 protein groups from a single cell, further highlighting the power of recent technologies using state-of-the-art instrumentation (Supplementary Fig. S6). Protein identification depth can be further improved using the same instrumentation through additional optimization of the chromatographic column, LC conditions, MS acquisition parameters, spectral library generation from bulk samples, and MS data processing workflows. For single-cell proteomics of multicellular plants, enzymatic cell wall digestion to generate protoplasts is currently unavoidable. However, this process is likely to alter the native cellular proteome. An important next challenge would be to develop fixation methods that preserve the native proteome while remaining compatible with protoplast isolation (Makar et al. 2025, Piga et al. 2025).

Acknowledgments

We thank SCIENION/Cellenion for their support with protoplast sorting and MS sample preparation using the cellenONE platform. We thank Robert Haensel-Hertsch and Olivia van Rey (CMMC Cologne, University of Cologne) for kindly providing access to their cellenONE platform.

Author Contributions

S.C.S. and H.N. conceived this study. S.C.S., A.H., and H.N. designed experiments. S.C.S., A.H., and C.K. conducted experiments. S.C.S. and C.K. analysed data. S.C.S. and H.N. wrote the manuscript.

Supplementary Data

Supplementary Data is available at *PCP* online.

Conflict of Interest

C. K. is an employee of Bruker. All other authors declare no conflicts of interest.

Funding

This project was supported by the Max Planck Society.

Data Availability

The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE partner repository with the dataset identifier PXD080545.

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Plant and Cell Physiology, 2026, 00(00),1–4. https://doi.org/10.1093/pcp/pcag138
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