Editorial

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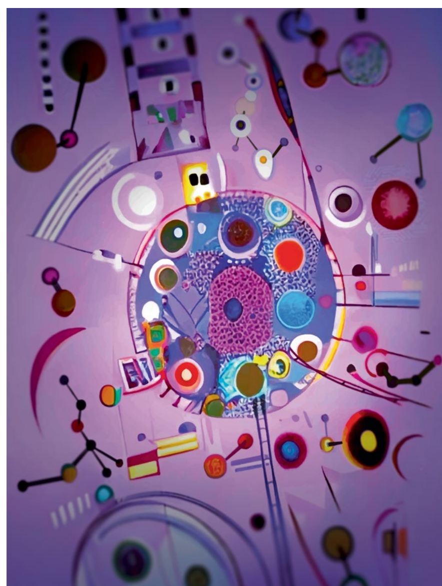
Single-cell proteomics: challenges and prospects

Single-cell proteomics is a challenging goal and an area of rapid methods development. This Focus issue highlights the many paths toward high-throughput, high-sensitivity measurements.

High-throughput sequencing of DNA and RNA in individual cells has revealed that molecular heterogeneity at the single-cell level is greater than previously realized. However, to fully capture functional heterogeneity, one must also sequence the proteome at single-cell resolution. Proteins are, after all, the primary functional molecules of the cell, and RNA abundances do not directly translate to protein abundances within cells. Furthermore, proteins contain post-translational modifications that are not captured by transcriptomics.

Single-cell proteomic technologies are in a more nascent state than genomic and transcriptomic technologies. Several hurdles must be overcome before we can comprehensively detect and quantify the thousands of different proteins in a cell. One limitation is that proteins, unlike DNA or RNA, cannot be amplified. Therefore, minimizing losses during sample preparation and handling, as well as during experiments, becomes a more significant concern. Another challenge is the dynamic range: proteins can be present at anywhere from one to ten million copies per cell, spanning a whopping seven orders of magnitude in dynamic range. Most current methods capture only a small portion of this, often missing low-abundance proteins — the 'dark proteome'.

A number of distinct strategies have been exploited to understand the proteomic makeup of cells. These include bottom-up mass spectrometry (MS)-based methods, in which the proteomic content of the cell is digested and analyzed; antibody-based methods, which typically target a small number of predefined proteins; imaging-based methods capable of detecting and providing the spatial context of proteins; and single-molecule sequencing methods. Implementing these



at the single-cell level, however, requires further optimization.

MS-based single-cell proteomics has had the most success thus far. Advanced automated sample preparation methods coupled with label-free or multiplexed data collection on highly sensitive instruments now allow researchers to routinely detect and quantify 1,000 to 1,500 proteins per cell. These numbers, however, pale in comparison to the total number of unique proteins and proteoforms in a cell. The current state of MS-based single-cell proteomics is reminiscent of the early days of next-generation sequencing. A Review by Spyros Darmanis and colleagues discusses how next-generation sequencing-based and MS-based methods for single-cell proteomics can provide complementary information. Targeted antibody-based methods that rely on sequencing of nucleic acid barcodes have the advantage of being able to measure multiple modalities from the same sample but do not provide the coverage that MS-based methods achieve. While these methods are being developed independently of each other, their potential integration into a multiomics approach is an exciting frontier.

As any rapidly evolving and expanding field, it is paramount for the proteomics community to establish and adopt standardized practices and metrics for better data alignment between laboratories. In a Perspective, Nikolai Slavov and other prominent researchers present initial recommendations on best practices for experimental design, data evaluation and interpretation, and data reporting for single-cell proteomics. While these recommendations are expected to evolve with further community discussions, we look forward to gauging community reaction and uptake, and recommend our authors consider and adopt these guidelines in their work.

A Comment from Matthias Mann and colleagues discusses technical challenges and emerging solutions for single-cell proteomics, including recent efforts to extract and analyze single cells from tissue samples with the spatial context in mind. The spatial context is a relevant consideration in single-cell proteomics, and an area where it is anticipated to have a significant impact is tumor heterogeneity. A Comment from Tami Geiger notes that characterizing the multiple cell types within a tumor requires accurate and deep analysis of thousands of heterogeneous cells. Understanding the spatial context within tumors can further help clinical stratification and treatment.

Another level of spatial context comes from protein localization within the cell. The subcellular localization and microenvironment of a protein have direct relevance to its biological function. A Comment from Lingjun Li and colleagues discusses recent advances in single-cell imaging MS, which can provide both spatial distribution and molecular information in an untargeted manner. Imaging MS can also identify and localize metabolites and lipids, providing much richer insight.

The next level of cellular resolution will target organelles, where many cellular processes are compartmentalized. While current capabilities are far from comprehensively profiling them, we need technologies capable of handling even smaller sample amounts, isolating and characterizing organelles of various sizes, and providing sensitive quantification. A Comment from James Eberwine and

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colleagues discusses this formidable technical challenge and outlines the methodological developments needed in this direction.

Another emerging technology that shows promise for single-cell proteomics is single-molecule sequencing. One such avenue popular for DNA sequencing is the use of nanopores: as linear DNA molecules pass through the nanopore, the blockade of ion current by each nucleotide is measured to decipher the sequence. Proteins have 20 different amino acids, plus post-translational modifications, which makes discriminating them unambiguously a bigger challenge. In a Comment, Keisuke Motone and Jeff Nivala talk about recent progress and challenges in protein nanopore technology.

Several protein sequencing or identification instruments are also under development, with many academic startups in this space. Many of these technologies immobilize intact or digested proteins to be sequenced using stepwise cleavage or Edman degradation, or detect them using different probes. While none of these technologies have a commercially viable product on the market yet, like nanopores, these have the potential to sequence low-copy-number proteins that MS-based methods tend to miss. Whether sequencing-based methods will become a practical solution for proteomics is yet to be seen. A Comment from Michael MacCoss and colleagues presents a compelling assessment of the challenges and costs associated with accurate and sensitive protein quantification in single cells by MS-based proteomics versus single-protein sequencing methods.

Finally, in our Technology Feature, journalist Vivien Marx interviews method developers in these different spaces to get a sense of where these technologies stand. The piece offers a closeup of an actively developing field.

These are exciting times for single-cell proteomics, with tremendous potential to unlock new biological insight into cellular structure and function. We look forward to seeing how these methods grow in the years to come and how they complement other omics technologies.

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