High-throughput single-cell proteomics quantifies the emergence of macrophage heterogeneity

The fate and physiology of individual cells are controlled by networks of proteins. Yet, our ability to quantitatively analyze protein networks in single cells has remained limited. To overcome this barrier, we developed SCoPE2. It integrates concepts from Single-Cell ProtEomics by Mass Spectrometry (SCoPE-MS) with automated and miniaturized sample preparation, substantially lowering cost and hands-on time. SCoPE2 uses data-driven analytics to optimize instrument parameters for sampling more ion copies per protein, thus supporting quantification with improved count statistics. These advances enabled us to analyze the emergence of cellular heterogeneity as homogeneous monocytes differentiated into macrophage-like cells in the absence of polarizing cytokines. We used SCoPE2 to quantify over 2,000 proteins in 356 single monocytes and macrophages in about 85 hours of instrument time, and the quantified proteins allowed us to discern single cells by cell type. Furthermore, the data uncovered a continuous gradient of proteome states for the macrophage-like cells, suggesting that macrophage heterogeneity may emerge even in the absence of polarizing cytokines. Our methodology lays the foundation for quantitative analysis of protein networks at single-cell resolution.