SurfaceScout™: A Chemoproteomic Platform for Comprehensive Profiling of Cell Surface Proteins and Macromolecular Uptake Mechanisms

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Background: Cell surface proteins play a central role as drug targets, uptake receptors and biomarkers, yet their systematic analysis remains challenging due to low abundance and poor solubility. Here, we describe the implementation of SurfaceScout™, a robust chemoproteomic platform for the quantitative profiling of cell surface proteomes at increased scale and reproducibility. SurfaceScout™ enables the identification of tissue– and disease-specific markers, therapeutic antibody targets and receptors for the targeted uptake of macromolecular therapeutics such as ASOs and siRNAs. Method: SurfaceScout™ combines selective biotinylation of surface N-glycans with a semi-automated enrichment and analysis workflow to quantify cell surface proteins. While mild conditions for cell surface labeling preserve cell integrity, the automation of key processing steps enhances reproducibility, scalability and throughput, enabling the analysis from relatively low cell numbers and sensitive cell types. The platform allows for insights into the composition of surface proteomes across cell types as well as mechanistic and uptake studies. Results: SurfaceScout™ was implemented as a semi-automated platform to achieve robust and scalable characterization of cell surface proteomes. In a representative study using HepG2 and HEK293 cell lines, approximately 600 annotated cell surface proteins were identified per cell line (using Surfy annotation), while more than 1,100 proteins were detected with over tenfold enrichment relative to background. Comparative analysis revealed cell type–specific surface proteins, reflecting distinct pharmacological target availability, and differential enrichment of proteins associated with receptormediated endocytosis and macromolecular transport, suggesting inherent differences in their uptake capacity for larger drug modalities such as ASOs and siRNAs. Conclusion: The platform robustly resolves biologically meaningful differences in surface accessibility, providing a quantitative basis for the rational design of targeted delivery strategies and antibody-based therapeutics. Due to its scalability and ability to capture dynamic changes, SurfaceScout™ is also suitable for larger-scale biomarker discovery and interrogation of macromolecular uptake mechanisms.