TECHNICAL NOTE

Comparing Single-Cell Intracellular Proteome with Traditional Phosphoflow

Bruker's Intracellular Signaling Omics reveals phosphoproteomic signaling networks in days, not weeks, with fully optimized and validated panels.

In this Technical Note we outline:

- How Bruker overcomes the limitations of traditional phosphoflow
- How Bruker eliminates the need for complex panel design steps of traditional phosphoflow
- How Bruker's platform can reveal multiple pathways to drug resistance
- How Bruker's single-cell proteomics predicts success of combination therapy

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Flow-based methods vs. Single-Cell Intracellular Proteome

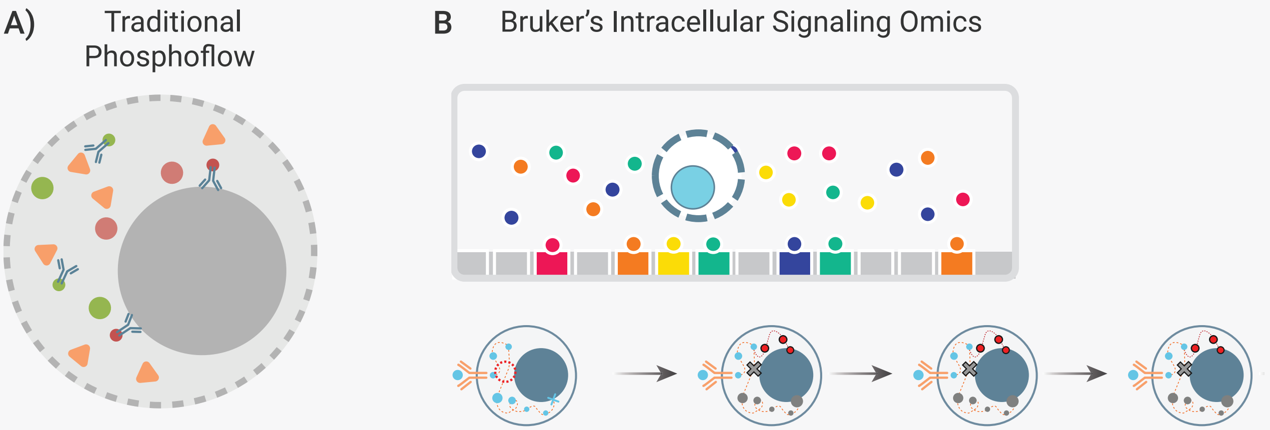

Figure 1 | A) Traditional phosphoflow is limited in the detection of complete functional signaling pathways and involves complicated workflows B) Bruker's barcoded microchambers provide a much larger capture area, enabling multiplexed phosphoprotein detection, and providing missing data layer that enables the development of effective combination therapies by identifying the mechanisms of drug resistance before it occurs.

The Importance of Functional Characterization in Cancer Therapeutic Resistance

While researchers often look to genomic markers to understand the tumor microenvironment and signaling pathways, functional adaptations can occur in the tumor that are unrecorded in their genomic signatures. It would be very difficult to provide a complete picture of the tumor's mechanisms of therapeutic resistance with genomics alone, however, as drugs targeted to these identified pathways have still resulted in drug tolerance due to functional adaptations. The Single-Cell Intracellular Proteome solution is therefore a critical tool for researchers studying tumor/protein signaling pathways and the bases of therapeutic resistance, providing complete functional characterization.

Bruker Overcomes the Limits of Traditional Phosphoflow

Current methods of identifying phosphoproteins, such as with traditional phosphoflow, have excessively complicated workflows and do not provide complete insights into the entire signaling network. Bruker's unique Single-Cell Intracellular Proteome solution targets 500-1500 individual cells per chip with 15+ analytes per single cell, uniquely measuring cellular communication via phosphorylation events, providing complete insights into the entire signaling network and revealing critical adaptive resistance pathways. Bruker Systems analyze up to four Single-Cell Intracellular Proteome chips simultaneously, connecting intracellular proteins back to each individual cell and unlocking the next level of resolution in cellular and immune research.

Prep, Run, Analyze

Single-cell intracellular proteome technology can provide insights on the full network of cellular signaling pathways. This can provide researchers invaluable data to overcome cancer therapy resistance and identify functional adaptive changes in cancer cells.

Comparatively, Bruker microchambers provide a much larger capture area for phosphoprotein detection compared to traditional phosphoflow-based methods, which use the cell itself as the capture area (Figure 1). To maintain accuracy in such a small capture area, traditional phosphoflow-based methods are typically limited to detecting 2-5 types of phosphoproteins. In contrast, Bruker's Single-Cell Intracellular Proteome solution is fully automated, allowing for highly-multiplexed detection of phosphoproteins (Figure 2).

Bruker's systems measure phosphoprotein intensity, thus enabling insights into intracellular pathways, disease progression, and mechanisms of therapy resistance. After loading your Single-Cell Intracellular Proteome chips into the IsoLight or IsoSpark, the entire process is automated, as Bruker's unique proteomic barcode relays each sample's data to the IsoSpeak software downstream to provide publication-ready data visualizations. The IsoLight and IsoSpark platforms with the Single-Cell Intracellular Proteome solution enables predictive intracellular discoveries, revealing true functional biology to accelerate research and medicine.

Bruker's chamber-based method enables high multiplexing of phosphoproteins concurrently vs. what can be reliably assayed with traditional phosphoflow

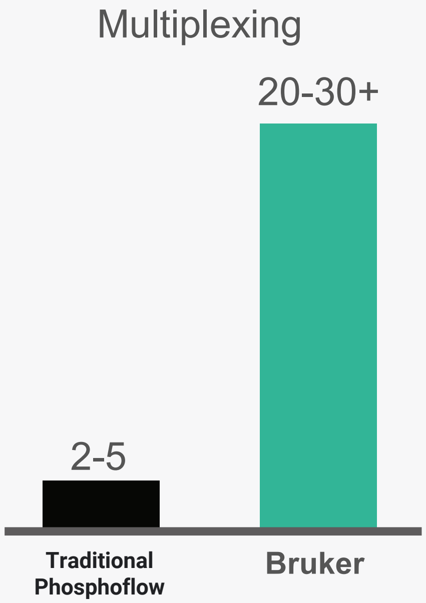

Figure 2 | Bruker's systems enable the detection of a broad range of phosphoproteins, enabling full characterization of cellular reponse.

Prep, Run, Analyze

Bruker overcomes the limitations of traditional phosphoflow panel development out of the box for immediate insights

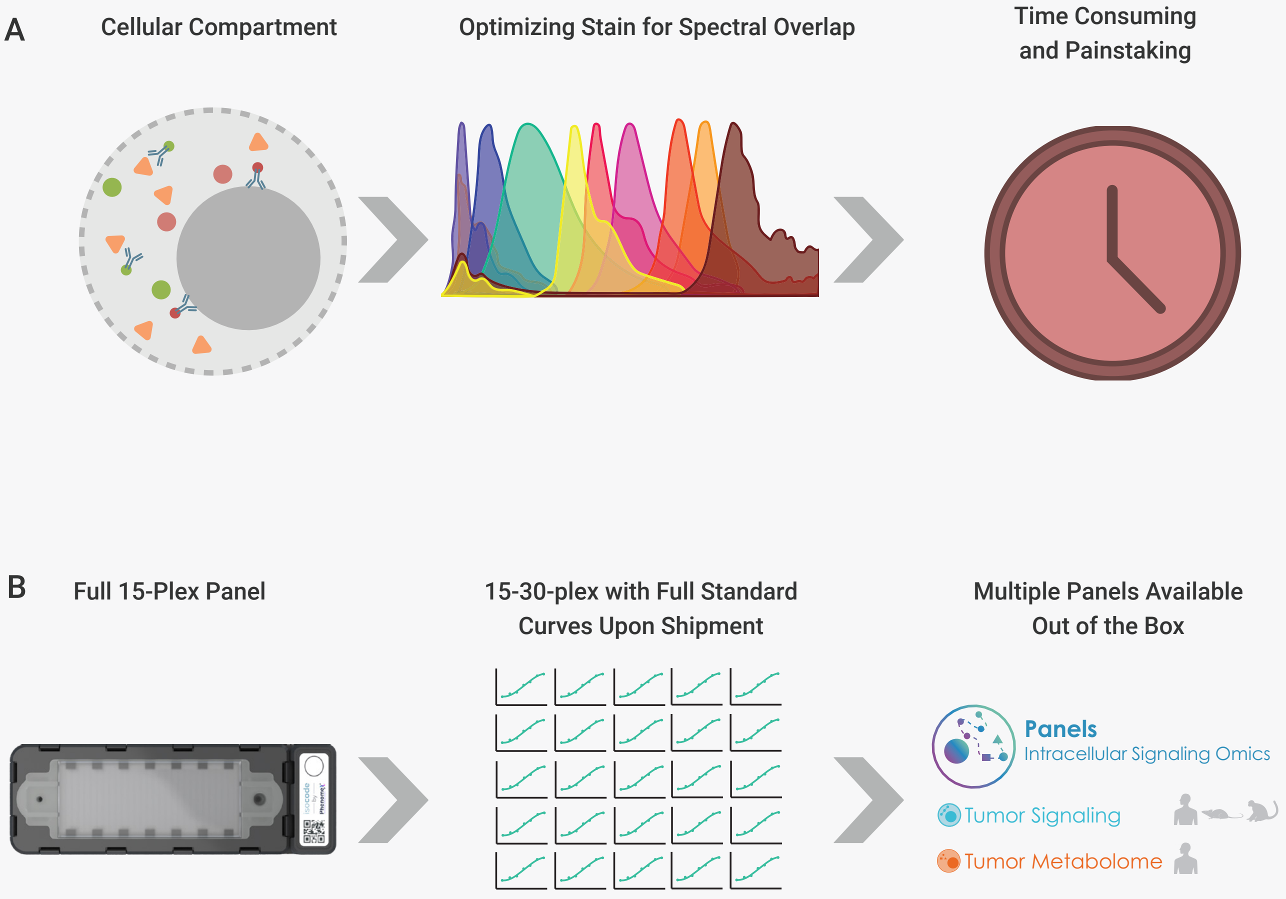

Figure 3 | A) Setting up a traditional phosphoflow assay involves filter and laser configurations, multiple titrations and iterations, and accounting for spectral overlap, leading to increased experiment time.
B) Out of the box, Single -Cell Intracellular Proteome is well-validated with sensitive and specific panels available to measure multiple types of signaling pathways.

Higher multiplexing and more specificity enables complete tumor cell pathway mapping

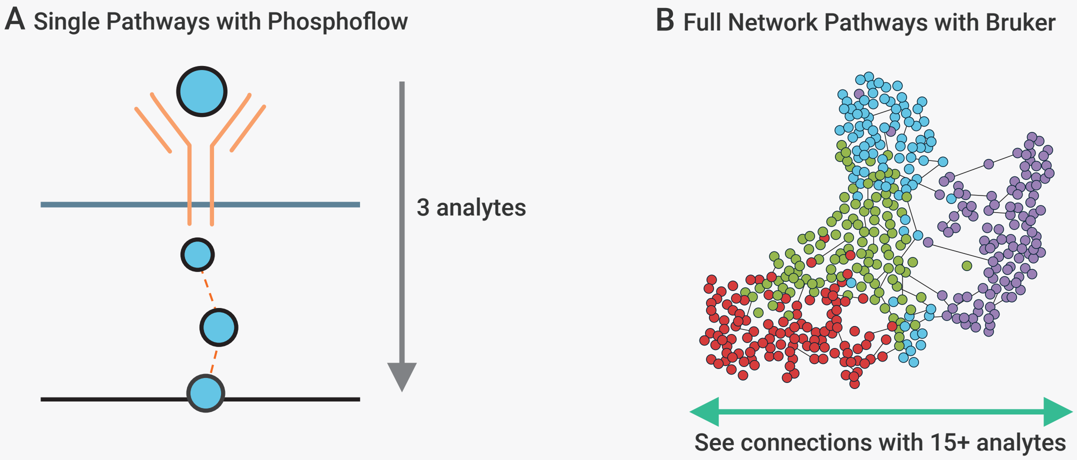

Figure 4 | A) It is difficult to fully characterize complete pathways to resistance with phospho-flow based methods, partially due to the limited numbers of analytes that can be detected simultaneously. B) Bruker's system provides the missing data layer that enables the development of effective combination therapies by identifying the mechanisms of drug resistance before it occurs.

Bruker Eliminates the Need for Complex Panel Design Steps of Traditional Phosphoflow

With traditional phosphoflow, panel design is a complicated and very involved iterative process before starting your experiments. First, a user would need to identify the laser and filter configuration of the flow cytometer. Next, the user is required to use a software tool to visualize the spectral overlap of fluorophores to try to minimize the signal overlap from other flourophores. Following, the user is required to optimize the combination of each antibody, requiring multiple titrations and iterations. Different Fluorophores need to be optimized based on low or highly expressed proteins. And additionally, different fluorophores are selected based on optimization of spectral overlap. This process is repeated until an optimal panel is identified (Figure 3a).

Bruker eliminates the need for complex panel design. The comprehensive system eliminates different conditions based on laser and filter configurations. Bruker's unique proteomic barcoding eliminates challenges related to spectral overlap, and panels are fully validated and optimized and available to use immediately, with no optimization by the researcher is required (Figure 3b). This means researchers are getting critical insights in days not months.

Create pre-clinical meaning: The full pathway map

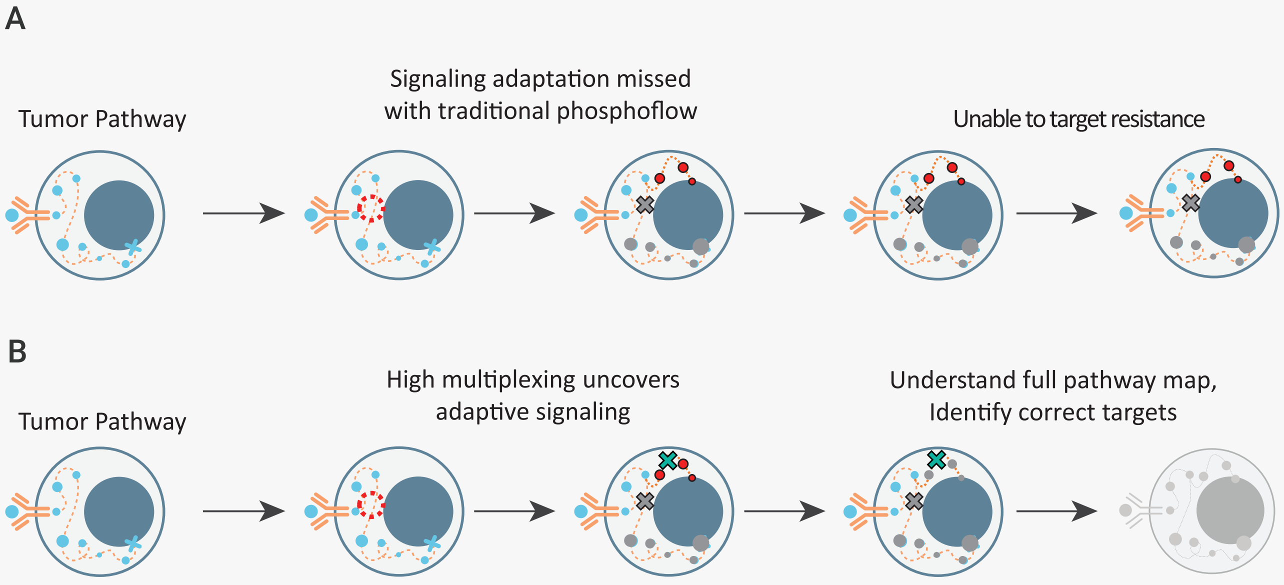

Figure 5 | A) Fewer concurrent pathways are able to be detected with traditional phosphoflow. B) Drugging and detecting multiplexed cancer driver pathways from each cell clarifies multiple pathway impacts of adaptive proteomic signaling networks, simultaneously. With Bruker, more concurrent pathways are revealed to treat resistance.

Bruker's Platform Reveals Multiple Pathways to Drug Resistance

Characterization of therapy resistance is essential to creating effective and curable courses of treatment, and the ability to define and measure each of the individual contributing signaling pathways is critical to this characterization. With traditional methods such as traditional phosphoflow, the inability to multiplex means that new panels must be designed in order to define multiple pathways, adding significant time and resources to the process. The ability to highly multiplex allows for a fuller picture of the cellular functional response to new therapies by enabling the capture of a full network of adaptations to therapy.

Bruker Complete Pathway Map Enables Targeted Therapeutic Discovery

Traditional flow-based methods detect a cancer signaling pathway once it has been activated, which becomes the target for treatment. Once resistance to the treatment develops, the tumor returns, and additional experimental runs must be developed and performed. Targeting the functional adaptations prior to activation has been an effective strategy for the prevention of resistance, and by highly multiplexing for 15+ phosphoproteins simultaneously, multiple resistance pathways can be detected to determine the most effective target (Figure 5).

Bruker's Single-Cell Proteomics Predicts Success of Combination Therapy

Using Bruker's Single-Cell Intracellular Proteome technology, researchers were able to identify a solution to overcome resistant cells by analyzing the differential responses in signaling coordination. They showed that drug resistance can proceed via a non-genetic (adaptive) mechanism that is activated within days of drugging. The measured adaptive response points to combination therapies that are tested *in vivo* and shown to halt tumor growth (Figure 6). This single-cell analytical approach appears to provide clinically actionable insights into designing combination therapy strategies for more effectively treating GBM patients.

Cancer Cell publication: Bruker single-cell proteomics led to successful combination therapy in Glioblastoma

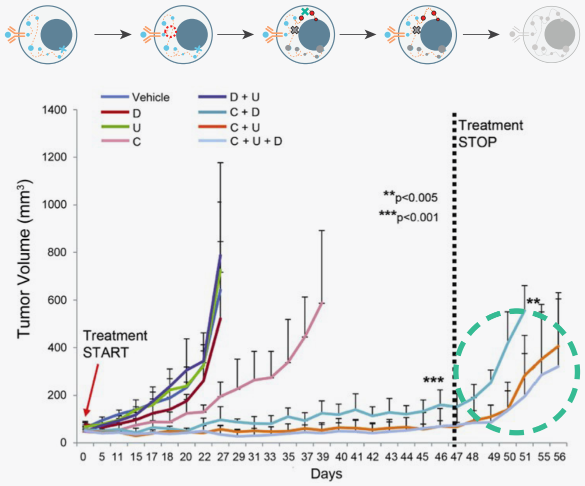

Figure 6 | Results for the seven monotherapy or combination therapies based upon the predictions from the Single Cell Intracellular Proteomics. All seven predictions proved correct. Dotted Circle: Bruker enables novel combination therapies to overcome tumor resistance.*.

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* Wei W, et al. Single-Cell Phosphoproteomics Resolves Adaptive Signaling Dynamics and Informs Targeted Combination Therapy in Glioblastoma. Cancer Cell, 2016.