FlashNote

Deep Protein Characterization Simplified: SampleStream-timsOmni-ProSight Native

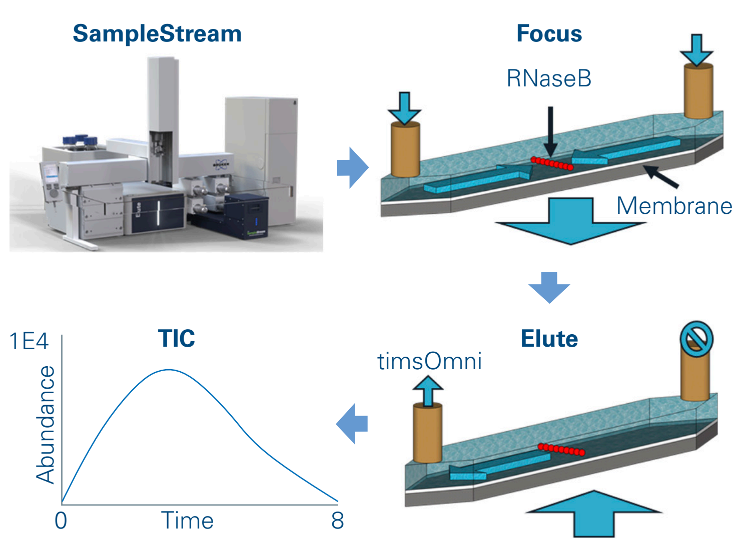
Top-down characterization of intact proteins often requires spectral averaging to build ion statistics. The SampleStream™ platform addresses this need by delivering buffer-exchanged samples to the detector over an extended and controllable period. When combined with the advanced fragmentation capabilities of timsOmni™ and the user-friendly ProSight Native data analysis, this creates a straightforward and powerful workflow for protein characterization.

The front-end challenge for intact protein characterization

Top-down experiments produce many fragment ions, but the limited number of ions detected per scan event makes complete protein characterization on a chromatographic timescale challenging. To overcome this, different approaches to front-end automation are needed to extend signal duration.

Automated buffer exchange with variable elution flow rate

The SampleStream platform, an online molecular weight cutoff (MWCO) filtration system, is an ideal complement to the timsOmni for deep protein characterization. After sample injection, clean solvent carries samples to the flow cell, where analytes are retained by the MWCO membrane while salts and buffer components are removed to waste. Subsequently, an elution flow delivers the buffer-exchanged and concentrated analytes to the detector. The elution flow rate can be adjusted from 1.6 to 300 μL/min while maintaining a constant peak volume. By modulating the elution flow rate, the sample peak can be extended over time (up to ~8 minutes). This extended signal duration allows the timsOmni to generate detailed and extensive fragmentation data of intact proteins using its array of fragmentation techniques. Following data collection, the ProSight Native platform processes intact protein spectra and searches fragmentation data to obtain precise proteoform assignments and fragment annotations.



As an integrated solution, this approach provides a powerful and seamless workflow, covering sample preparation to spectral collection to data analysis, advancing the tools available for intact protein characterization.

Guillaume Tremintin[1], Phil Compton[2], Ken Durbin[3]; [1]Bruker Daltonics GmbH & Co. KG, Bremen, Germany; [2]Integrated Protein Technologies; [3]Proteinaceous, Inc., Evanston, IL, United States.

SampleStream Parameters

5 µL of RNase B at 100 ng/µL in 100 mM NH4Ac was injected into the SampleStream flow cell using a 5 kDa MWCO membrane and 30% ACN w/ 0.1% FA as the system solvent. The sample was pushed to the flow cell with 50 µL at 280 µL/min. Buffer exchange was accomplished with 250 µL at 280 µL/min. Sample was eluted to the mass spectrometer with a high flow step (15 µL at 30 µL/min) followed by a low flow step of 30 µL at 3 µL/min during which time data acquisition occurred.

Fragmentation Data Acquisition

The 13+ of each of 2 glycoforms visible in the intact spectrum were selected and subjected to ECD fragmentation in the Omnitrap® platform.

Data Analysis

Data were imported into ProSight Native and analyzed using the interactive deconvolution and the top-down search workflow workflows. Intact masses were analyzed using a modified THRASH algorithm. The effectiveness of the SampleStream for sample desalting and adducts minimization was using a mirror plot (Figure 1). MS^2 spectra from approx. 3 min to 7.5 min were averaged and the resulting fragment ions were compared to the proteoform sequence of RNase B modified with the Man8 glycan (Hex(8) HexNac(2)), resulting in 74% sequence coverage as well as localization of the glycan.

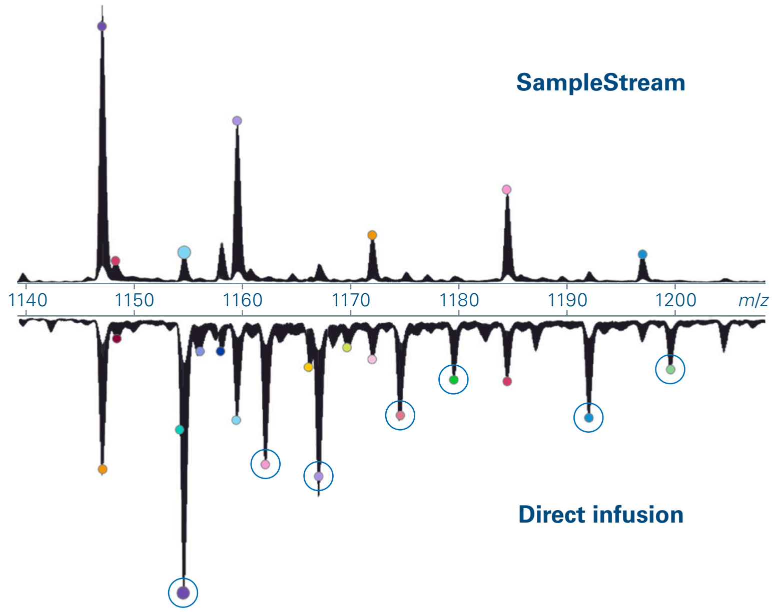
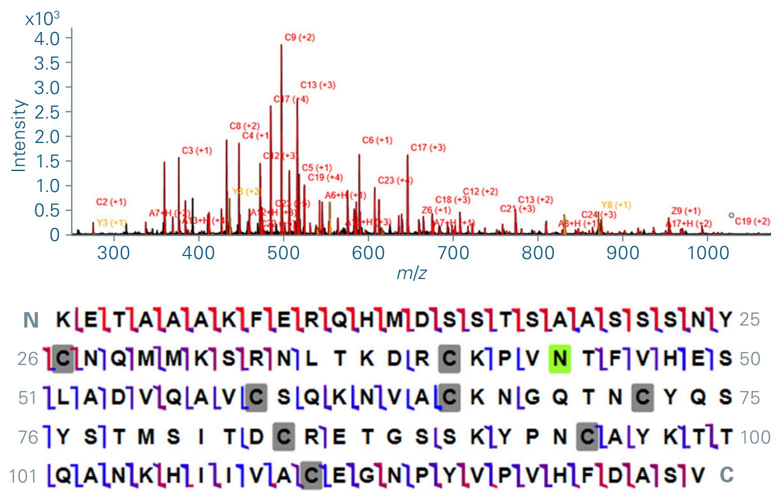


Figure 1. **SampleStream significantly reduces adducts.**
Intact proteoforms were determined by ProSight Native. The different proteoforms for the 13+ of RNAase B are all shown here. Blue circles highlights adducts removed after comprehensive SampleStream desalting.



Mono. Mass	Matching Ions	N-terminus Explained %	C-terminus Explained %	Total Residues Explained %
15375.84	217	61.0%	56.9%	74.0%

Figure 2. **Deep characterization of glycosylated proteoforms.**
ProSight Native annotated ECD MS2 spectrum of RNase B (top) with the corresponding fragmentation map (bottom).

Bruker Switzerland AG

Fällanden · Switzerland
Phone +41 44 825 91 11

Bruker Scientific LLC

Billerica, MA · USA
Phone +1 (978) 663-3660

ms.sales.bdal@bruker.com – www.bruker.com

Learn more at **www.bruker.com/timsomni**