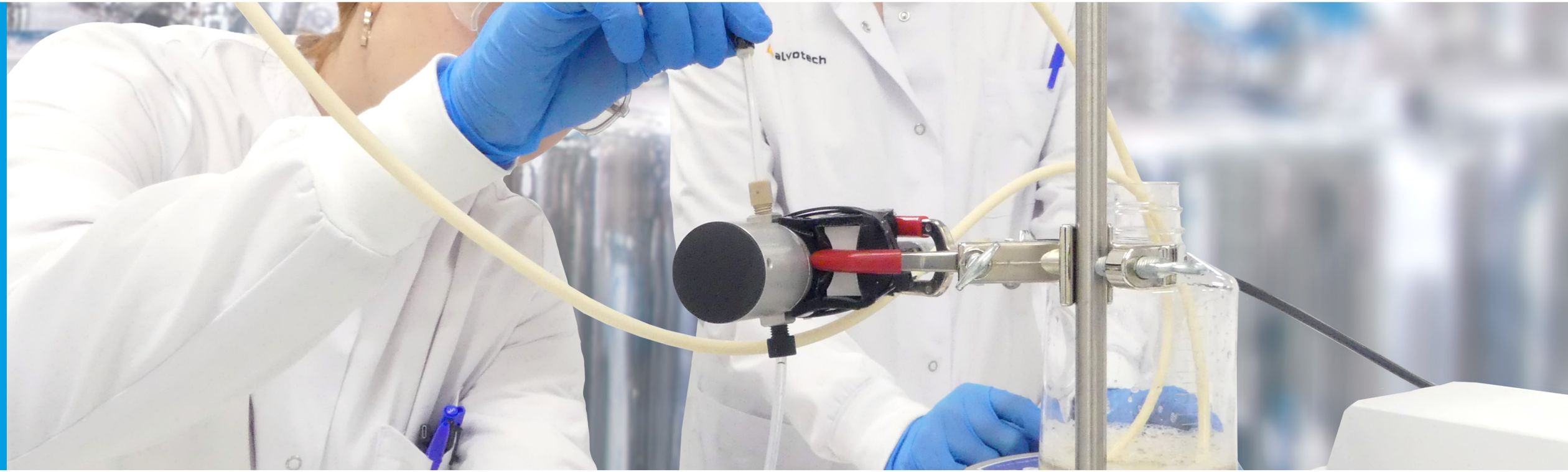
Raman downstream process measurement of biosimilars

Success story Alvotech's testing of the protein purification process during ultrafiltration/diafiltration (UF/DF)



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Challenges & results

Customer challenges

Downstream processes experience rapid composition changes. Consequently, process development in the downstream phase necessitates a Process Analytical Technology (PAT) that accommodates reduced volumes and shorter cycle times. However, efficiency improvements for downstream applications have been slow to materialize due to several technological challenges, such as reliance on extractive analytical methods for composition measurement, slow response times, insufficient nominal ranges, background interferences, and more. The increasing complexity of purification steps, driven by intensified upstream processes, further underscores the need for innovative downstream solutions.

Summary of results

Endress+Hauser's Raman spectroscopy systems enhance downstream processing (DSP) for biosimilar production by enabling precise, real-time monitoring of the protein purification process during ultrafiltration/diafiltration (UF/DF). Benefits can include:

- **Cost savings:** reduced labor, buffer solution, and offline analysis costs lead to significant savings
- **Faster processing time:** real-time monitoring and adjustment of the UF/DF process eliminate process pauses, resulting in streamlined operations.
- **Process optimization:** instant analytical results enable proactive decision-making, enhancing overall efficiency.

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"The beauty of Raman spectroscopy lies in its versatility. Applied in-line to UF/DF processes, it transforms operations by enabling real-time detection of protein aggregation and monitoring of concentration changes. This not only enhances process control, but it is a game-changer for optimizing bioprocessing efficiency, ensuring top-tier product quality and regulatory compliance."

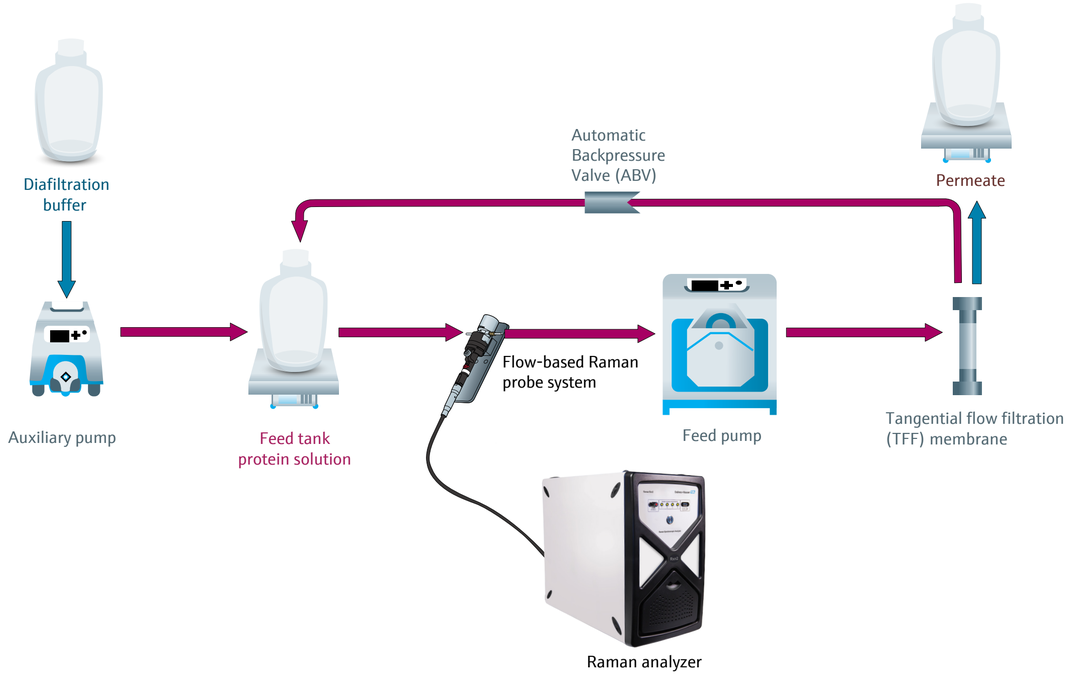
Adrianna Milewska, PhD,
PAT Lead
Alvotech

UF/DF downstream processing explained

The UF/DF downstream stage of bioprocessing typically involves the following steps:

1. The initial concentration step involves using ultrafiltration membranes to concentrate the protein solution. During this stage, excess salts, small molecules, and water are removed to achieve a more concentrated protein solution.
2. Diafiltration buffer is continually added while small molecules such as solvents, ions, and water are removed through ultrafiltration. The goal of this step is to exchange the process buffer for a final formulation buffer and reduce small impurities, such as ions, to acceptable levels.
3. A second concentration step is then performed to achieve the final desired protein concentration. These steps are integrated into a single process (UF/DF) that effectively concentrates the drug substance and exchanges the buffer before proceeding to the fill-finish stage.

Incorporating Raman into the UF/DF process



Traditional UF/DF systems generally have the capability to monitor system pressures, flow rates, and both feed and permeate volumes. However, a UF/DF system integrated with a Raman probe could potentially monitor monomer and aggregate levels, as well as the concentrations of buffer excipients in-line.

- **Initial concentration phase:** the volume of the feed tank decreases as water, small molecules, and ions are filtered out by the tangential flow filtration (TFF) membrane. The protein cannot pass through the membrane and is therefore retained within the system.
- **Diafiltration phase:** the volume of the feed tank is maintained at a constant level by continuously adding diafiltration buffer while simultaneously filtering out the process buffer.
- **Final concentration phase:** at this point, the buffer in the feed tank has been completely exchanged for the diafiltration buffer. The protein solution can now be concentrated to the desired level.

Downstream bioprocess limitations during UF/DF

Alvotech was looking to test solutions to possible process limitations related to the use of tangential flow filtration (TFF) methods that can occur during UF/DF, including:

- **Product quality and efficacy:** aggregation risks compromising monoclonal antibody quality, affecting pharmacokinetics and therapeutic effectiveness.
- **Patient safety:** aggregates can trigger immune responses, underscoring the importance of minimal levels in the final product for patient safety.
- **Process control and optimization:** real-time aggregation monitoring in TFF enables swift issue detection, optimizing process conditions faster in development stages, and minimizing downstream adjustments.
- **Product loss and yield:** TFF monitoring prevents aggregate formation, reducing losses and maximizing product yield during downstream processing.
- **Consistency in manufacturing:** ensuring batch-to-batch consistency is crucial for biopharmaceutical safety and efficacy, guaranteeing a reliable manufacturing process.
- **Economic considerations:** early aggregation detection minimizes costs by reducing the need for additional testing and wait times
- **Regulatory compliance:** stringent guidelines mandate comprehensive monitoring of biopharmaceuticals for approval and commercialization.

These limitations can cause process measurement uncertainties and require frequent post-process manual adjustments. These adjustments consume valuable time and resources and have the potential to compromise product quality and efficacy.

Our solution

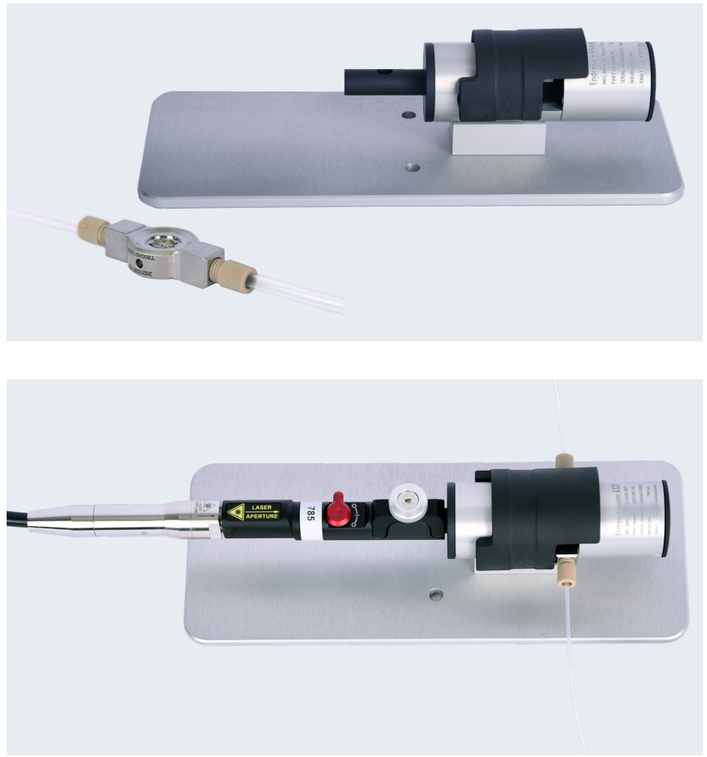
Alvotech chose Endress+Hauser to test in-line Raman spectroscopy in upstream and downstream process development. The selected Raman system is tailored for downstream flow paths, leveraging signal amplification and low-noise technology to deliver faster results. It consists of the following components:

- Raman Rxn2 analyzer
- Rxn-10 probe
- Raman flow assembly

The Raman flow assembly is a specialized tool designed for biopharmaceutical labs and process development in purification and perfusion spaces, with the potential to scale up to cGMP manufacturing.

The Raman flow assembly comprises:

- A reusable optic (micro flow bench) connected to an Rxn-10 probe, which has no product contact and is precisely tuned for specific flow cell and sample conditions.
- A micro flow cell that interfaces with the micro flow bench, allowing the sample to flow within it. The micro flow cell can be sterilized by approved methods and is suitable for either reuse or disposal after use.



(Top) micro flow cell and flow bench components; (bottom) Raman flow assembly connected to an Rxn-10 probe

Raman Rxn2 analyzer

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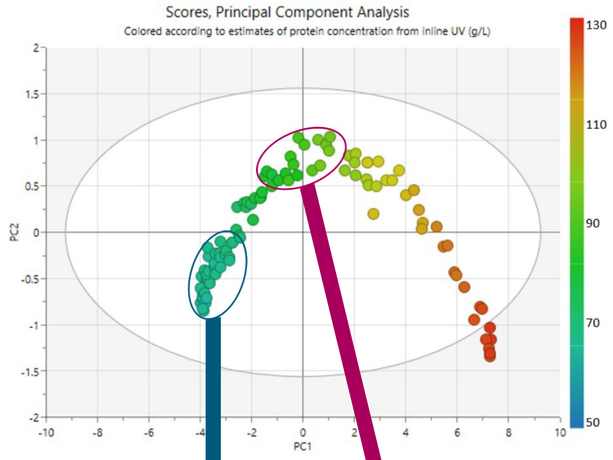
Raman data preview

Raman inline measurements during UF/DF support optimizing and advancing the efficiency of biopharmaceutical production.

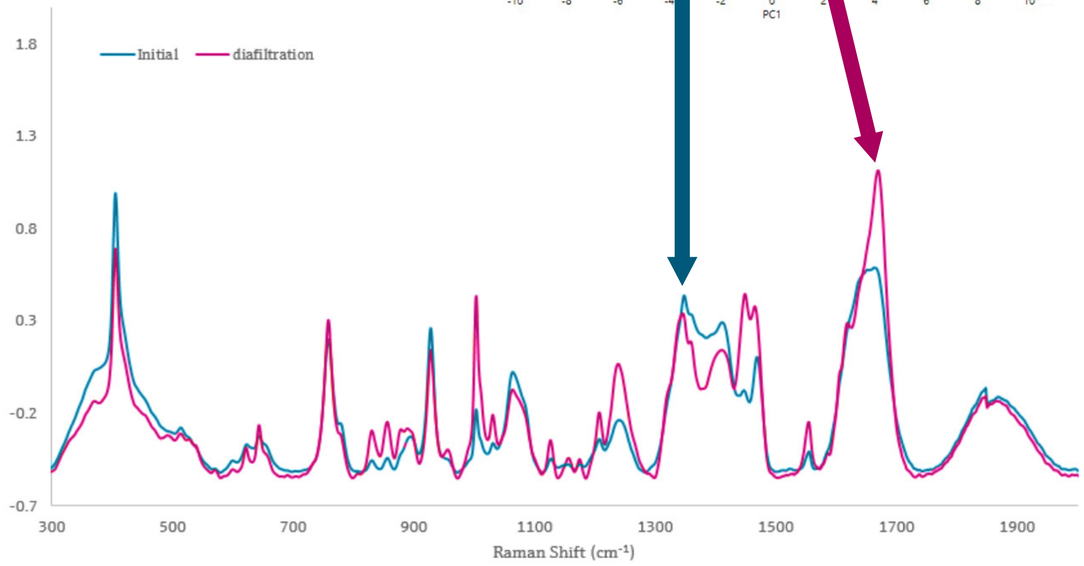
With standard preprocessing, Raman has shown the ability to track the process progression overall, with multiple markers throughout the fingerprint and a sharp deviation during diafiltration.

Further data analysis involving chemometrics modeling is then required to determine the contributions of protein aggregation. Once complete, this analysis will enable rapid feedback to enhance process understanding, improve process time, facilitate quick adjustments, reduce cost, and ensure the final product meets rigorous quality standards.

Evidence of protein-specific conformational changes at a localized cluster in the principal component analysis scores space is observed where multiple grab samples were obtained.



Raman spectral overlay at the diafiltration stage reveals strong well-resolved peaks consistent with protein specific interactions. The spectral overlay at right is taken from average spectra in the circled regions on the top graph in the scores space, where the blue spectra represents Raman shifts during initial concentration and the magenta spectra represents shifts during diafiltration.



Benefits of real-time measurement

The Endress+Hauser Raman system provides Alvotech with real-time monitoring, which can result in rapid process optimization and precise control over the protein purification process.

By integrating a Raman flow cell directly into UF/DF processes, it is possible to detect aggregation and continuously monitor protein concentration.

Immediate access to in-line Raman data, with an enhanced signal-to-noise ratio, facilitates the development of a predictive model based on concentration data from both in-line and at-line UV absorbance spectrometers, along with off-line size-exclusion chromatography (SEC). This streamlined set up optimizes protein purification, resulting in significant time and cost savings.

Benefits

The following capabilities and associated benefits can be gained by real-time access to accurate inline Raman process measurements, including:

- **Predictive modeling:** Develop and integrate predictive models based on in-line Raman data for proactive decision-making and process adjustments, optimizing outcomes.
- **Aggregation detection:** Detect protein aggregation in-line using Raman flow cell technology and predictive models, allowing early risk identification and mitigation.
- **Faster processing time:** Eliminate waiting times associated with off-line high performance (HP)-SEC analysis, promoting continuous operation and enhancing productivity.
- **Reduced manual intervention:** Minimize the need for manual adjustments post-process, streamlining operations and improving final concentration accuracy.
- **Real-time monitoring:** Continuously monitor protein and buffer excipient concentration.

In-line Rxn-10 probe connected to a Raman flow assembly monitoring protein purification during UF/DF

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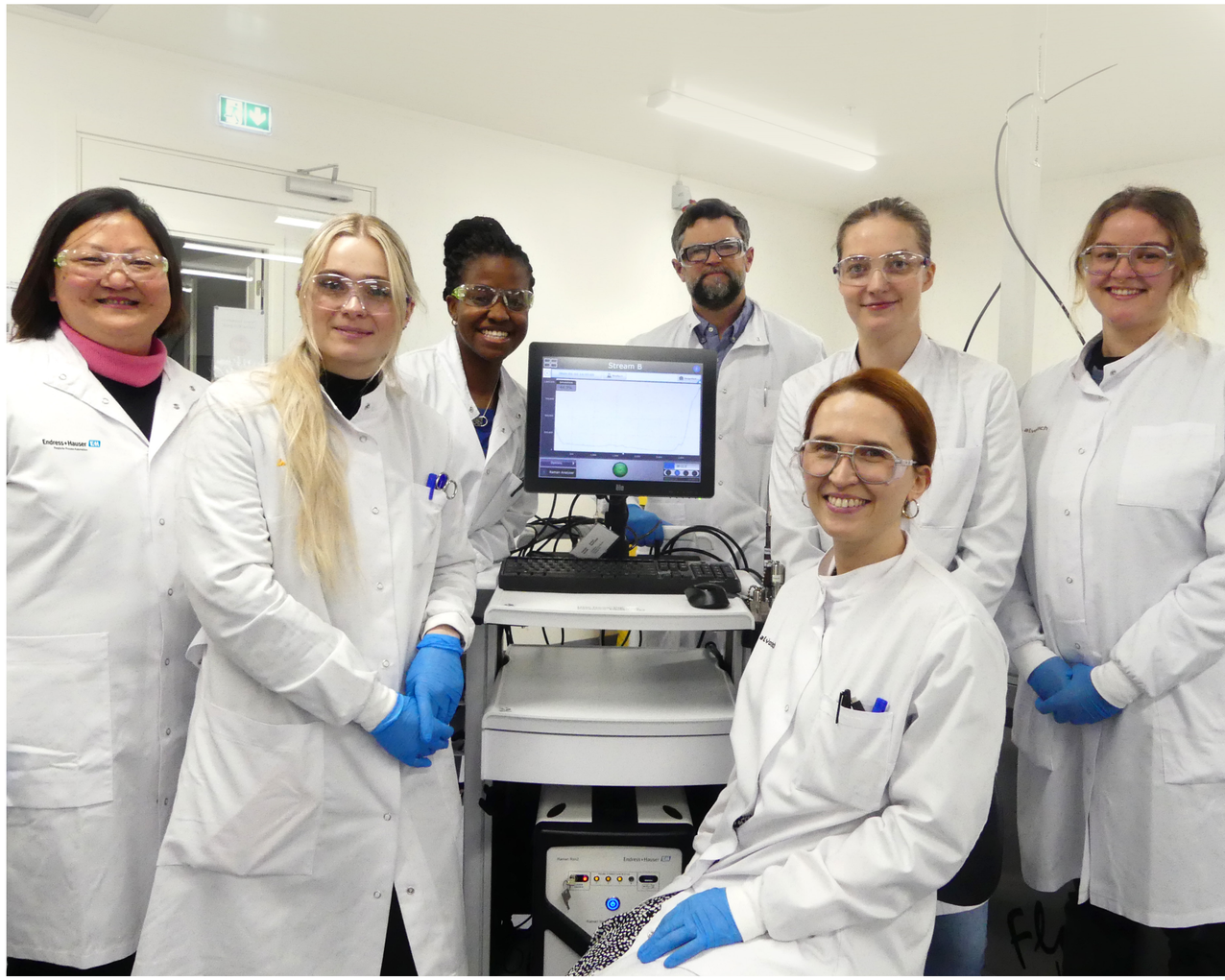
Conclusion

In the biopharmaceutical industry, recent advancements in Raman flow cell technology address the speed requirements of downstream processes by optimizing Raman collection for low-turbidity, low volume samples. Alvotech, with the assistance of Endress+Hauser's Raman system, has achieved valuable insights into protein processing during UF/DF. This enhanced monitoring has the ability to increase bioprocess efficiency, shorten cycle times, and improve product quality for the company's manufacturing operation.

"Downstream processes often get fragmented due to long waits for off-line results, slowing down development timelines. Implementation of the in-line Raman flow cell into operations promises a more efficient approach, effectively changing downstream development dynamics."

Julia Karitas Helgadottir
DSP Scientist
Alvotech



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About Alvotech

Headquartered in Reykjavik, Iceland, Alvotech is an integrated biopharmaceutical company dedicated to the development and production of high-quality biosimilar medicines on a global scale. Their mission is to enhance the health and quality of life for patients worldwide, striving to broaden access to proven treatments for various diseases.

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