

# Model calibration

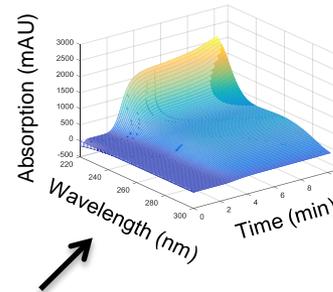
The patented method describes a versatile and powerful approach to controlling continuous chromatographic protein purifications. By leveraging optical spectroscopy in conjunction with chemometric methods, a wide range of separation challenges can be addressed.

The initial steps are related to calibrating a chemometric model for process monitoring in a single column setup:

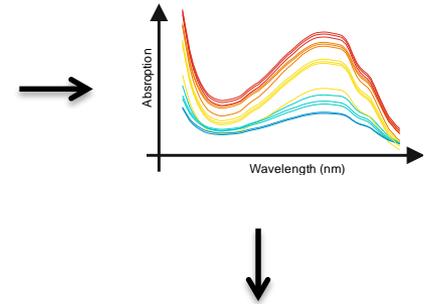
- 1) Calibration runs are performed
- 2) while spectroscopic data (2a) is recorded and fractions (2b) are collected
- 3) The fractions are analyzed with suitable off-line analytics
- 4) Spectroscopic data and fraction analysis are mathematically synchronized
- 5) Based on the data, a chemometric model is calibrated

Brestrich N, Sanden A, Kraft A, McCann K, Bertolini J, Hubbuch J. Advances in inline quantification of co-eluting proteins in chromatography: Process-data-based model calibration and application towards real-life separation issues. *Biotechnol Bioeng* 2015; 112:1406–16.

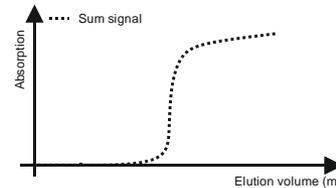
## 2a) Spectroscopic data



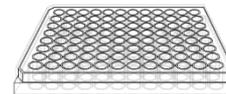
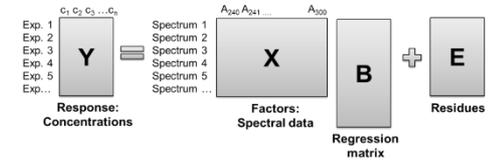
## 4) Synchronization



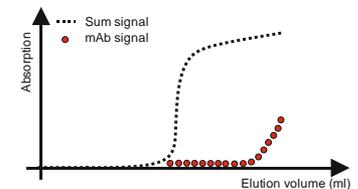
## 1) Calibration runs



## 5) Model calibration



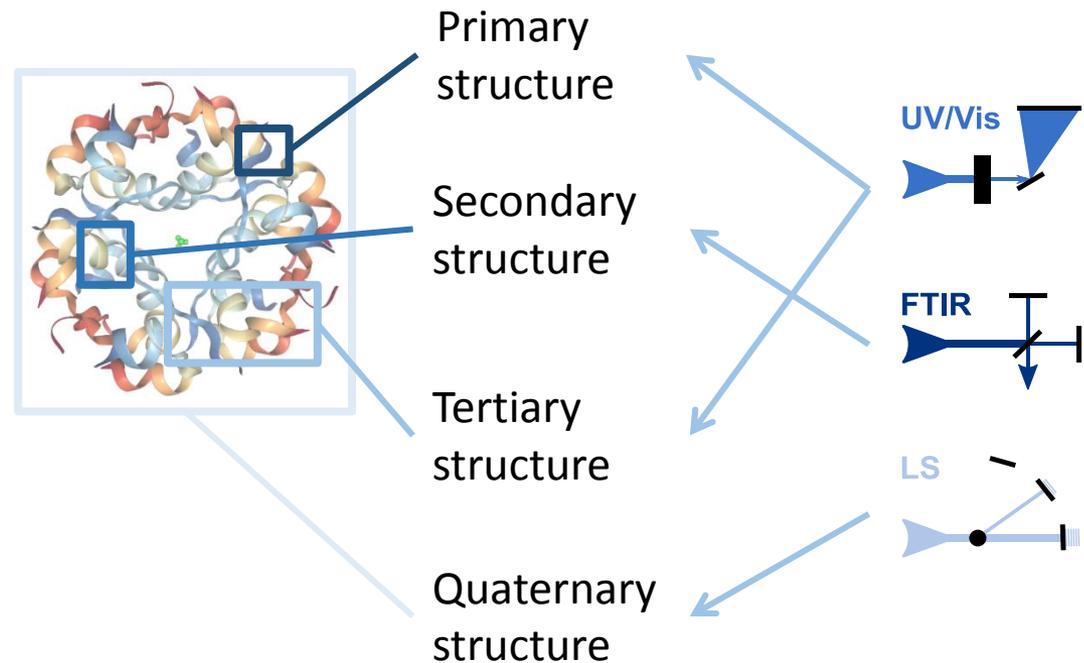
## 2b) Fraction collection



## 3) Fraction analysis

# Sensor selection

At KIT, the advantages of different sensors were investigated. Different spectroscopic methods allow to monitor the different structural levels in proteins. Similar sensors are also useful for monitoring small molecules and conjugation reactions. Research has shown that a knowledge-based sensor selection on a case-to-case basis can provide important information on the process and simplify process monitoring.

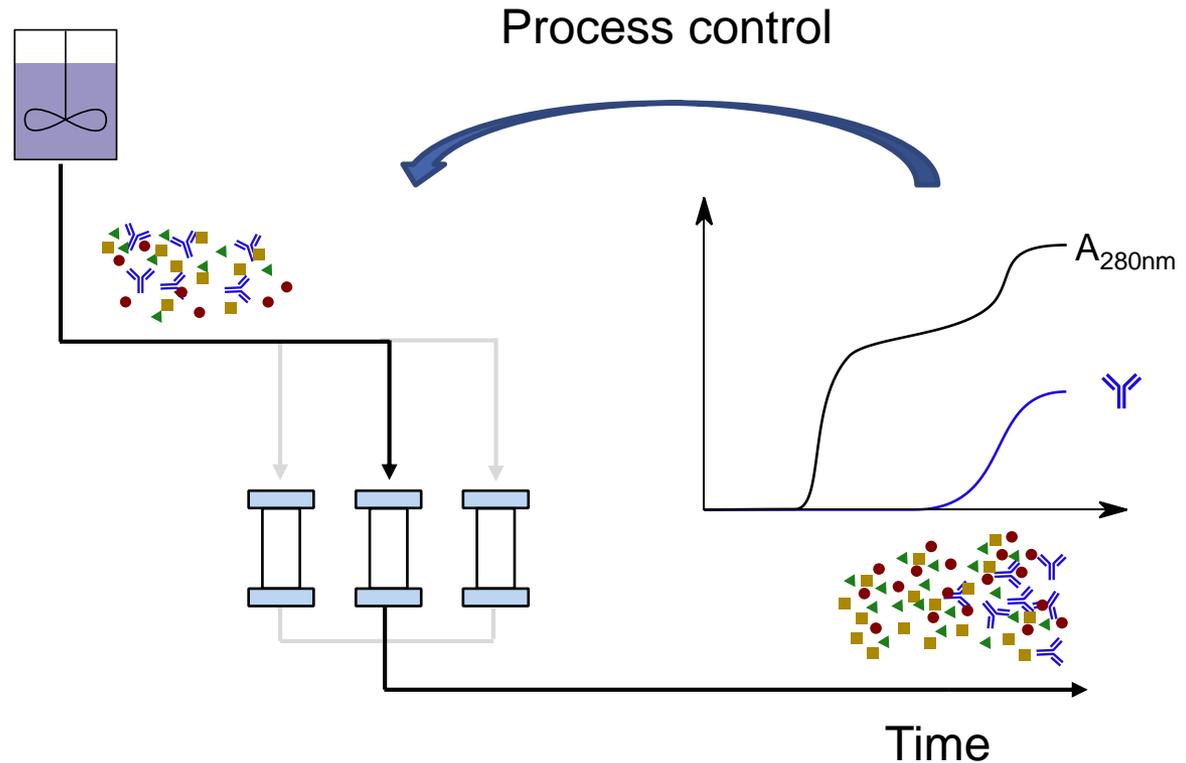


Rüdt M, Briskot T, Hubbuch J. Advances in downstream processing of biologics – Spectroscopy: An emerging process analytical technology. J Chromatogr A 2017; 1490:2–9.

# Continuous chromatography

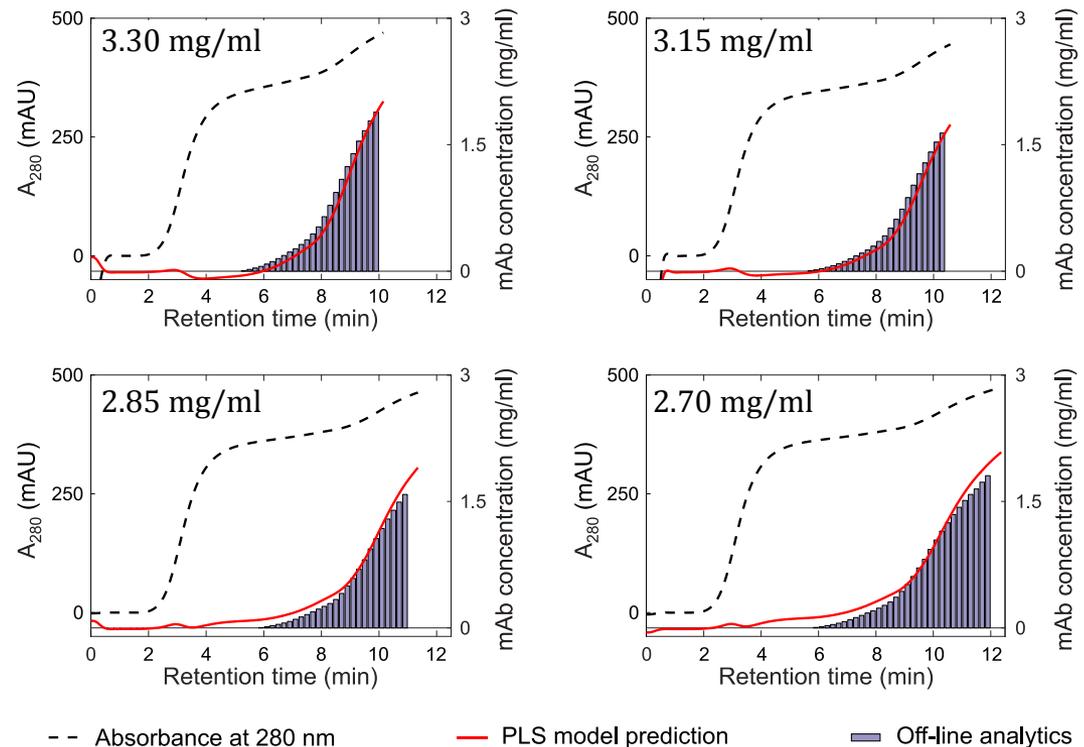
Especially for continuous processes, analytics close to the process can lead to major improvements. It can help to prevent process deviation and maintain a stable steady-state. Furthermore, process drifts may be registered early and thus, a loss in product minimized.

Our patent claims the application of spectroscopy for the control of continuous chromatography processes.



# Example 1: Protein A load control

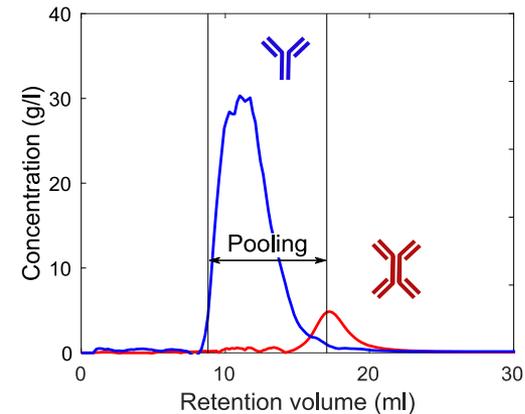
One of the most frequently used chromatography steps for mAbs is a Protein A capture. Generally, this process is run on predefined load volumes. At KIT, a dynamic control of the load phase was realized. The load step was automatically terminated at certain predefined breakthrough concentrations. This makes this technique interesting for continuous chromatography where the breakthrough protein is loaded on a subsequent column.



Rüdt M, Brestrich N, Rolinger L, Hubbuch J. Real-time monitoring and control of the load phase of a protein A capture step. *Biotechnol Bioeng* 2017; 114:368–73.

# Example 2: Controlling the preparative separation of mAb/HMW

Frequently, the product also needs to be separated from HMW impurities. We previously showed that spectroscopic techniques allow to differentiate between mAbs and HMWs. An automatic pooling decision was based on the predicted purities from the chemometric model. The measured pool purities by off-line analytics closely corresponded to the purities predicted by the chemometric model.

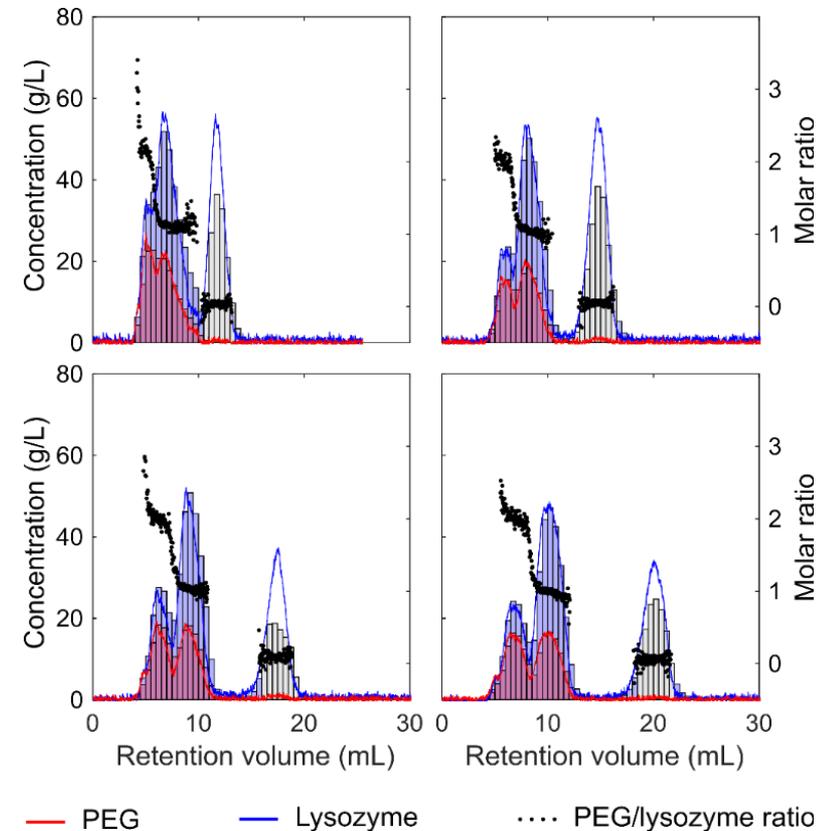
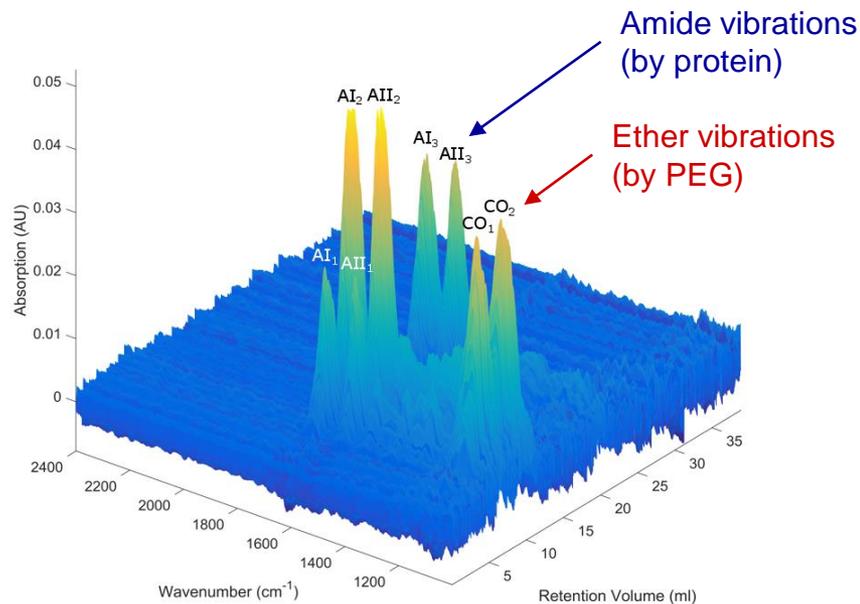


Method	Pool purity [%]
In-line estimation	94.4
Off-line analytics	94.2

Brestrich N, Rüdert M, Büchler D, Hubbuch J. Selective protein quantification for preparative chromatography using variable pathlength UV/Vis spectroscopy and partial least squares regression. Chem Eng Sci 2018; 176:157–64.

# Example 3: Monitoring the separation of PEGylated protein species

PEGylation is a frequently used protein modification to increase protein stability and its hydrodynamic radius. Monitoring the chromatographic steps with conventional methods is however difficult. Thus, the feasibility of applying FTIR spectroscopy was shown. With a chemometric model, the PEGylation degree could be monitored in-line.



Großhans S, Rüdert M, Sanden A, Brestrich N, Morgenstern J, Heissler S, Hubbuch J. In-line Fourier-transform infrared spectroscopy as a versatile process analytical technology for preparative protein chromatography. *J Chromatogr A* 2018; 1547:37–44.