Chemometric methods to enhance spectra quality and evaluate data obtained by a novel laser-based IR transmission setup for protein analysis

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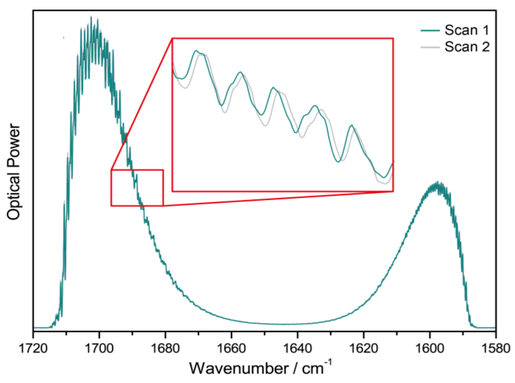
Introduction

In combination with advanced instrumentation technology, chemometrics has demonstrated to be a valuable tool for analytical methods. In the analysis of protein secondary structure by infrared spectroscopy, the use of the novel External Cavity-Quantum Cascade Lasers (EC-QCL) as light source has shown to provide a significant improvement of performance of the method. However, in spite of being commercially available, these light sources still suffer from imperfections in the tuning mechanism introducing high noise level due to shifts in the mode-hop fine structure of the emission curve within consecutive scans, leading to deviations in the final absorbance spectrum. Here correlation optimized warping (COW) was applied to eliminate high noise levels in absorbance spectra obtained by QCL-IR spectroscopy.

Furthermore, to showcase the potential and quality of the IR absorbance spectra obtained by QCL-IR spectroscopy, dynamic changes of proteins secondary structure in aqueous solution were studied at varying pH values and across a wide concentration range. Exposure of β-sheet rich proteins to 2,2,2-trifluoroethanol (TFE) leads to formation of nonnative α-helical structures. This fast transition is succeeded by gradual formation of intermolecular β-sheet aggregates. In this work the β-aggregation in alcohol-denaturated α-chymostrypsin was monitored by using a EC-QCL based IR transmission setup. Then, multivariate curve resolution based on alternating least squares (MCR-ALS) was used for analysis of spectral profiles of the temporal transition between α-helices and intermolecular β-sheets.

Noise Reduction

Reference and sample spectrum acquisition

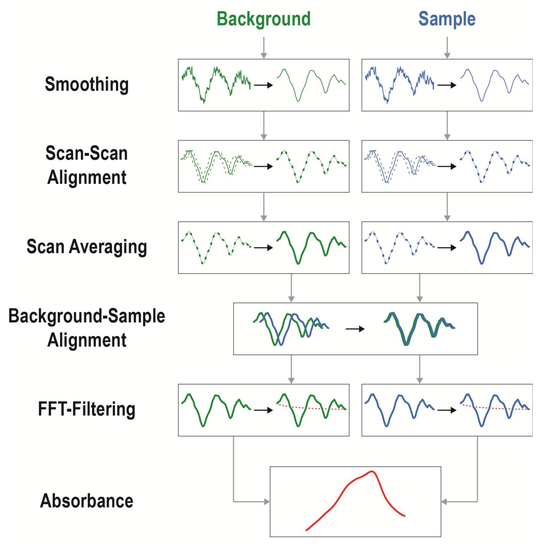


Small fluctuations in the single beam spectra lead to considerable noise in the corresponding absorbance spectrum

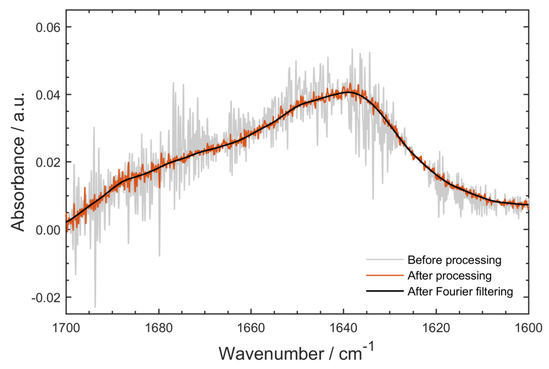
Data treatment routine based on several proceding steps allows to reduce the noise level of the absorbance spectrum. The key is the use of COW, which utilizes inherent mode-hop structures for scan-to-scan alignment.

Noise level is significantly reduced by aligning consecutive scans of one measurement prior to averaging, as well as the background with the sample single beam spectrum. The residual noise is removed by applying Fourier filtering

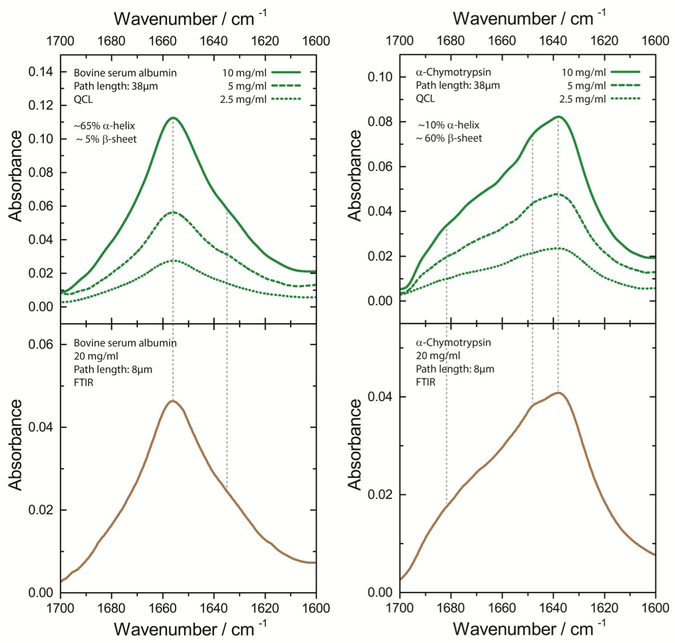
Data processing



Absorbance spectrum



Protein Spectra



QCL-based IR transmission measurements have been successfully employed to identify characteristic spectral features of proteins with different secondary structures.

Protein spectra acquired by EC-QCL transmission measurements show excellent agreement with absorbance spectra recorded by FT-IR spectroscopy.

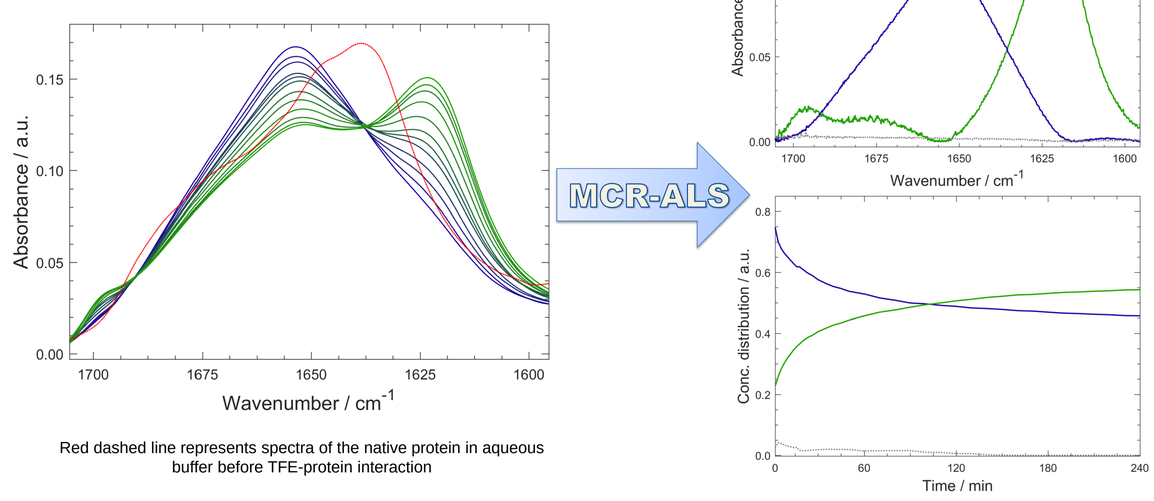
The combination between advanced technology and suitable data processing allowed to identify spectral feature of protein at concentration as low as 2.0 mg mL^{-1}

- Financial support was provided by the Austrian research funding association (FFG) under the scope of the COMET programme within the research project “Industrial Methods for Process Analytical Chemistry - From Measurement Technologies to Information Systems (imPACts)” (contract #843546).
- Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET)

Monitoring β-Aggregation

MCR-ALS modelling

Time-dependent IR of 20 mg mL^{-1} α-chymotrypsin in 50% TFE/buffer show the gradual β-aggregation.

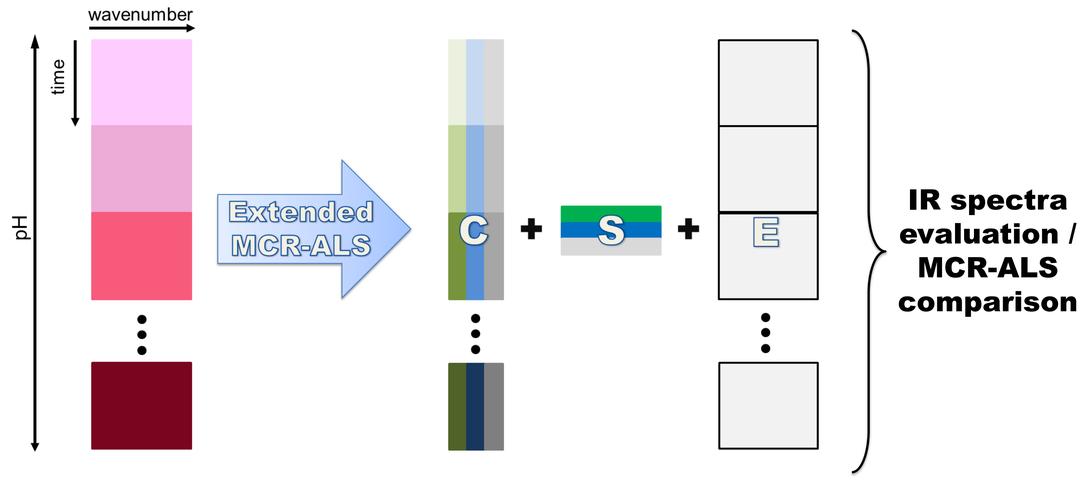


Red dashed line represents spectra of the native protein in aqueous buffer before TFE-protein interaction

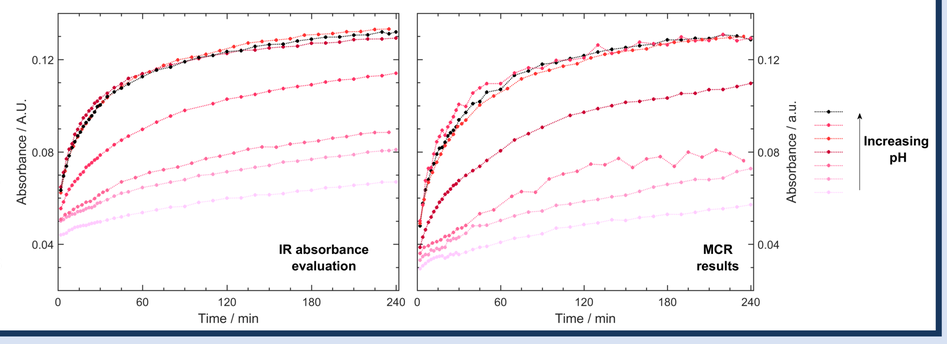
MCR-ALS was employed for obtaining the spectral profiles relating to the components involved in the secondary structure change.

pH dependence of β-aggregation

TFE-induced formation of intermolecular β-sheets of 20 mg mL^{-1} aCT in 50 % TFE/buffer solution was investigated in the range of pH 5.8–8.2.



The temporal profiles clearly show the strong pH dependence of the β-sheet formation since the change of absorbance is higher at elevated pH values



Conclusions & Outlook

The application of chemometrics enabled the use of EC-QCL light sources for IR transmission spectroscopy analysis. This procedure was successfully employed for monitoring time-dependent changes of protein secondary structure. Furthermore, MCR-ALS was used for obtaining reliable spectral information about the components involved in the secondary structure change.

Exploiting these advantages, this methodology will be applied to flow through measurements, as well as for the analysis of thermal protein denaturation.

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