Development and Optimization of a Mid-IR laser-based Mach-Zehnder Interferometer Setup for Dispersion Spectroscopy of Proteins

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Introduction

Mid-infrared (mid-IR) spectroscopy is an important analytical technique widely used in a variety of scientific fields. It allows for rapid, label-free investigation of chemical compounds in any form of matter (gas, liquid, solid), providing information about their functional groups or structure and allowing for molecule specific detection. The development of quantum cascade laser (QCL), a powerful and coherent source of mid-IR radiation, opened many new possibilities and approaches for spectroscopic sensing, triggering advancements in mid-IR spectroscopy.

Measurements of the changes in refractive index (dispersion) rather than absorption of the investigated molecules is one alternative approach to classical laser-based spectroscopic techniques. Dispersion sensing offers information equivalent to absorption spectroscopy and even though the phase shift measurements are experimentally more challenging than conventional absorption measurements, they are increasingly implemented because of multiple advantages, i.e. they are immune to source intensity fluctuations and offer high dynamic range for chemical detection. A number of methods for dispersion sensing of gaseous samples has been developed based on QCLs, while only few studies investigated the application of dispersion spectroscopy to liquid-phase samples [1,2].

In this work, an external-cavity quantum cascade laser (EC-QCL)-based Mach-Zehnder interferometer setup for dispersion and absorption spectroscopy of liquid-phase samples was developed and optimized for spectra acquisition in the challenging protein spectral region. The robust sampling technique based on attenuated total reflectance (ATR) was employed in the setup. Two proteins in aqueous solutions with different secondary structure were used as analytes – bovine serum albumin and β-lactoglobulin. The functionality of the setup was demonstrated and assessed by collecting dispersion and absorption spectra of proteins and performing qualitative and quantitative analysis.

Dispersion vs. Absorption Spectroscopy

Dispersion spectroscopy measures changes in refractive index (or phase shift), whereas the absorption spectroscopy measures the attenuation of intensity of light.

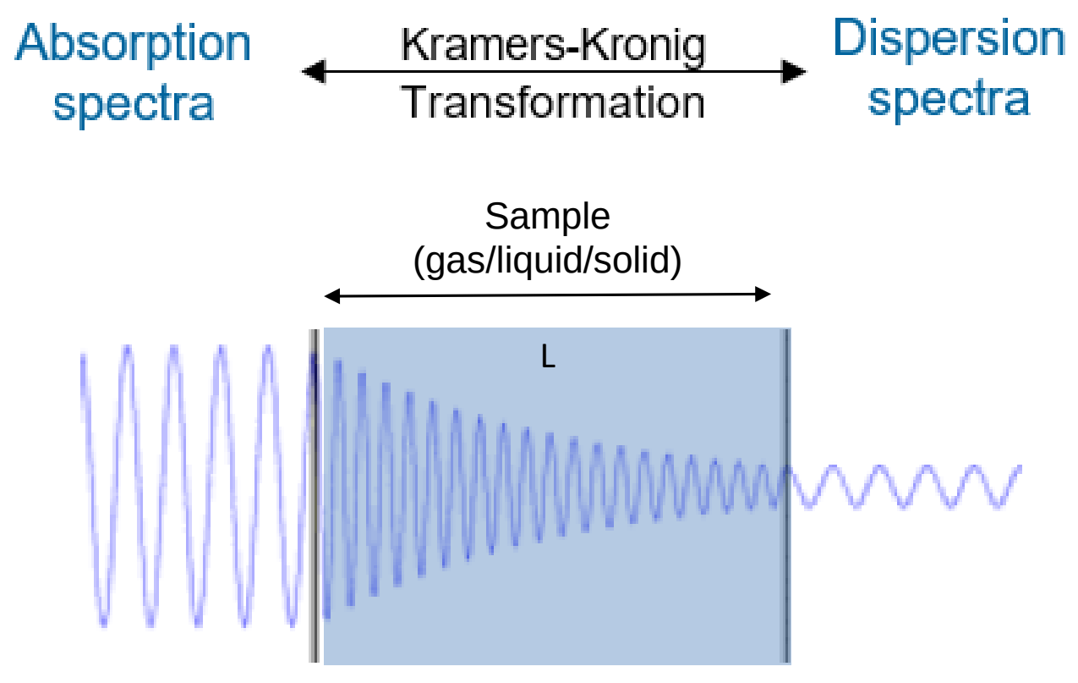
- Detection based on interferometric approach and coherent laser sources (QC lasers)
- Method established for gas-phase samples
- *Novel approach* for liquid-phase samples

$$\bar{n}(\tilde{\nu}) = n(\tilde{\nu}) + i\kappa(\tilde{\nu})$$

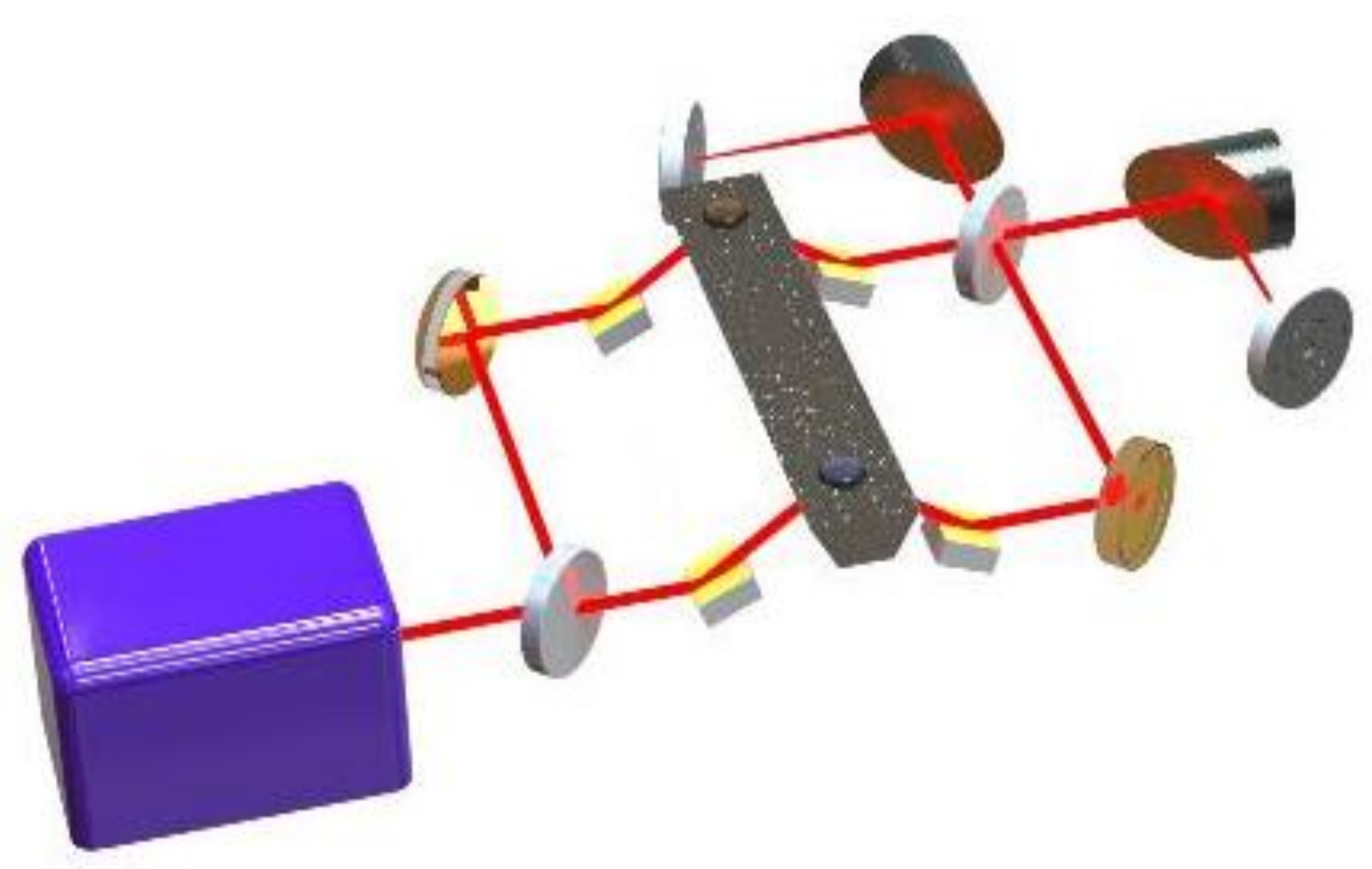
$$\Delta\varphi = \frac{2\pi}{\lambda}(n - n_0)L$$

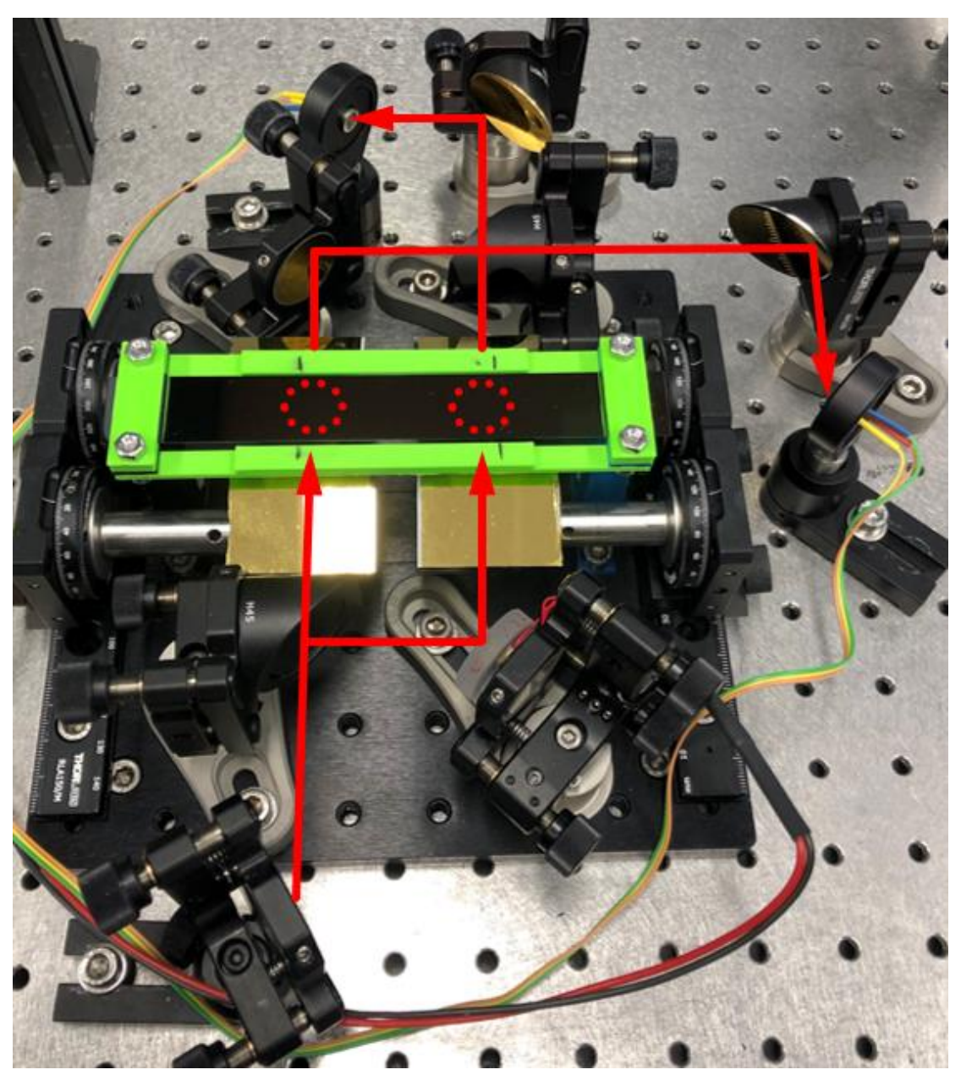
Advantages of dispersion spectroscopy:

- ✓ Immune to power fluctuations
- ✓ Linear output with concentration
- ✓ Baseline-free



Experimental Setup

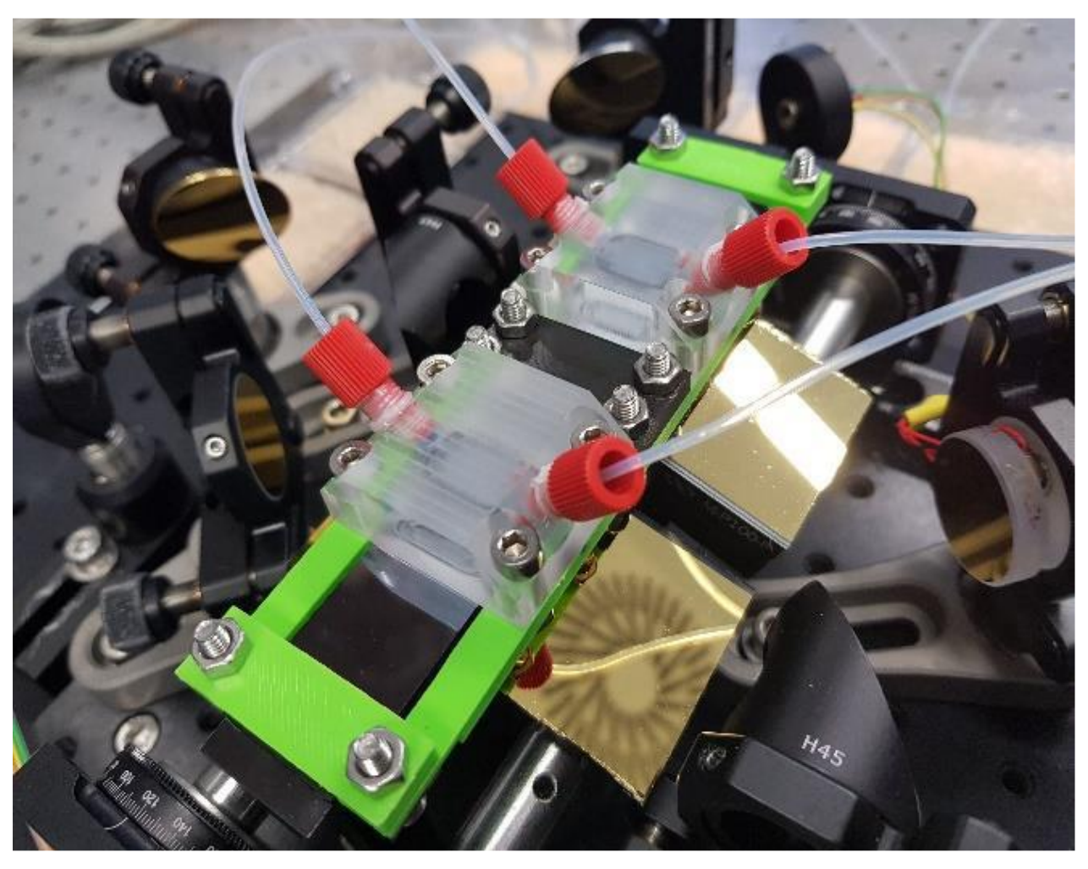




- Laser-based mid-IR spectroscopy was performed using an external-cavity quantum cascade laser with a tuning range between 1730–1565 cm^{-1}, covering the amide I region of proteins.
- Mach-Zehnder interferometer (MZI), known as an excellent instrument for sensitive phase shift detection, was employed in the setup. The interference signal allows to retrieve the information about the relative phase shifts, caused by the refractive index n of the absorbing sample introduced in one of the arms of the MZI.
- A trapezoidal single-bounce silicon (n=3.4) crystal was incorporated inside the MZI enabling the ATR sampling technique.
- A piezoelectric drive attached to one of the mirrors is used to adjust the mirror position to compensate for the difference between optical path lengths of the two interferometer arms.

Measures to improve sensitivity:

- *thermal stabilization* of the setup components and internal environment realized by a water-cool breadboard,
- *dry air purge* of the sealed setup led to significant reduction of disruptive water vapor content in the protein region,
- *reproducible sample injection* and sample control by two custom-made flow cells mounted on top of the crystal at the point of light-sample interaction.



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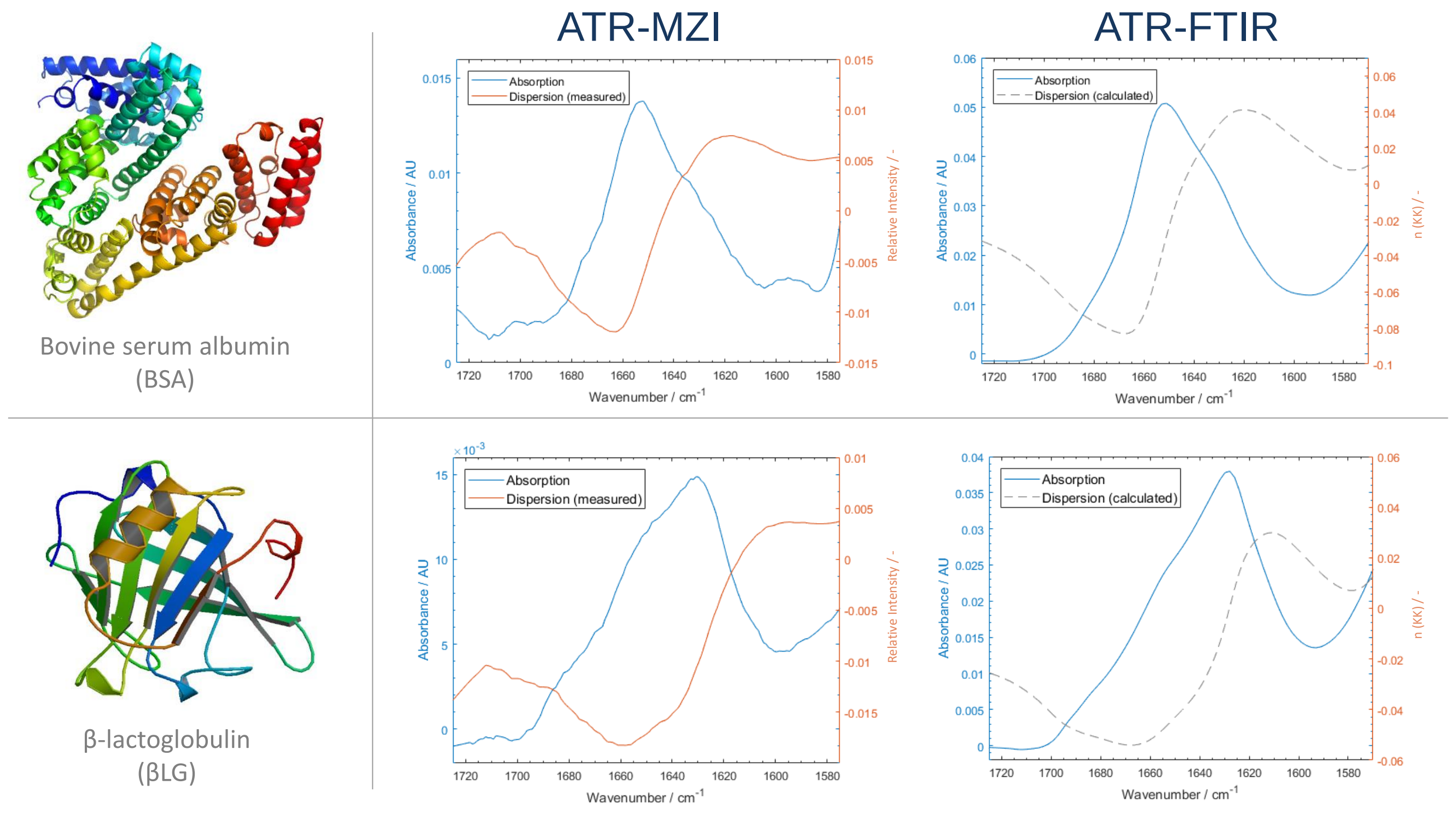
Dispersion Spectra of Proteins

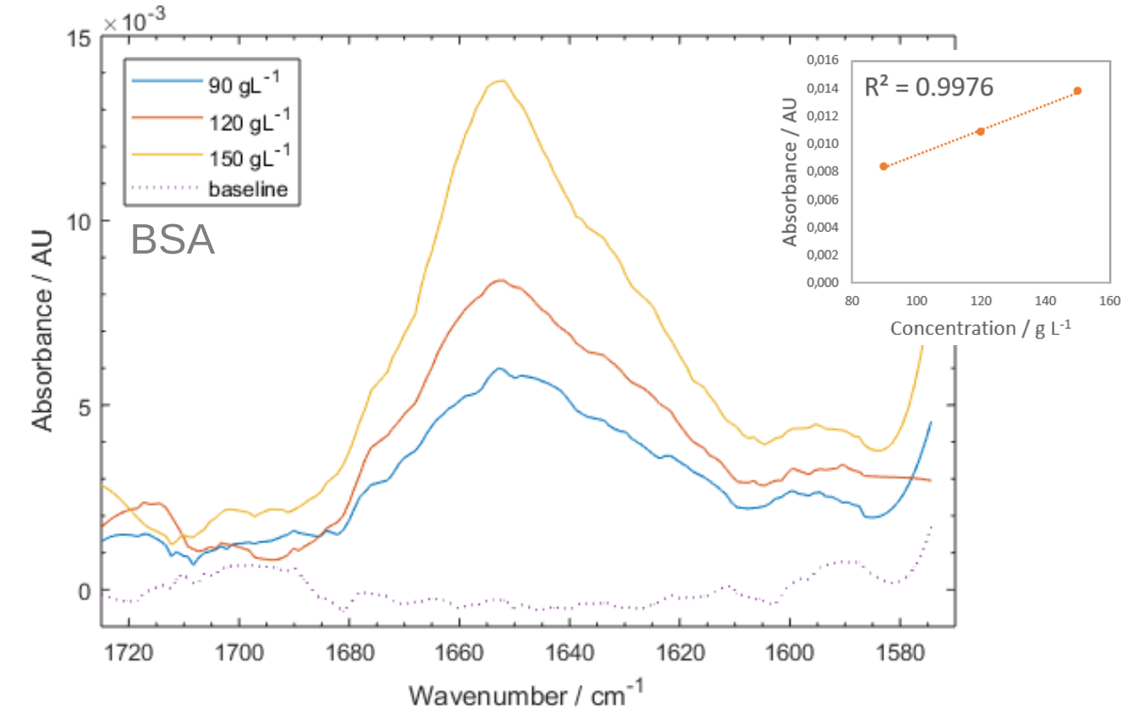


- Regions of the rapid change in the refractive index (anomalous dispersion) coincide very well the respective absorbance bands.
- The recorded (MZI) dispersion spectra were compared to the Kramers-Kronig transformed reference (FTIR) absorption spectra. The shape and positions of the measured spectra agree very well with the calculated spectra, proving the ability of the dispersion method and the developed setup to discriminate between the proteins with different secondary structures.

Quantitative Results

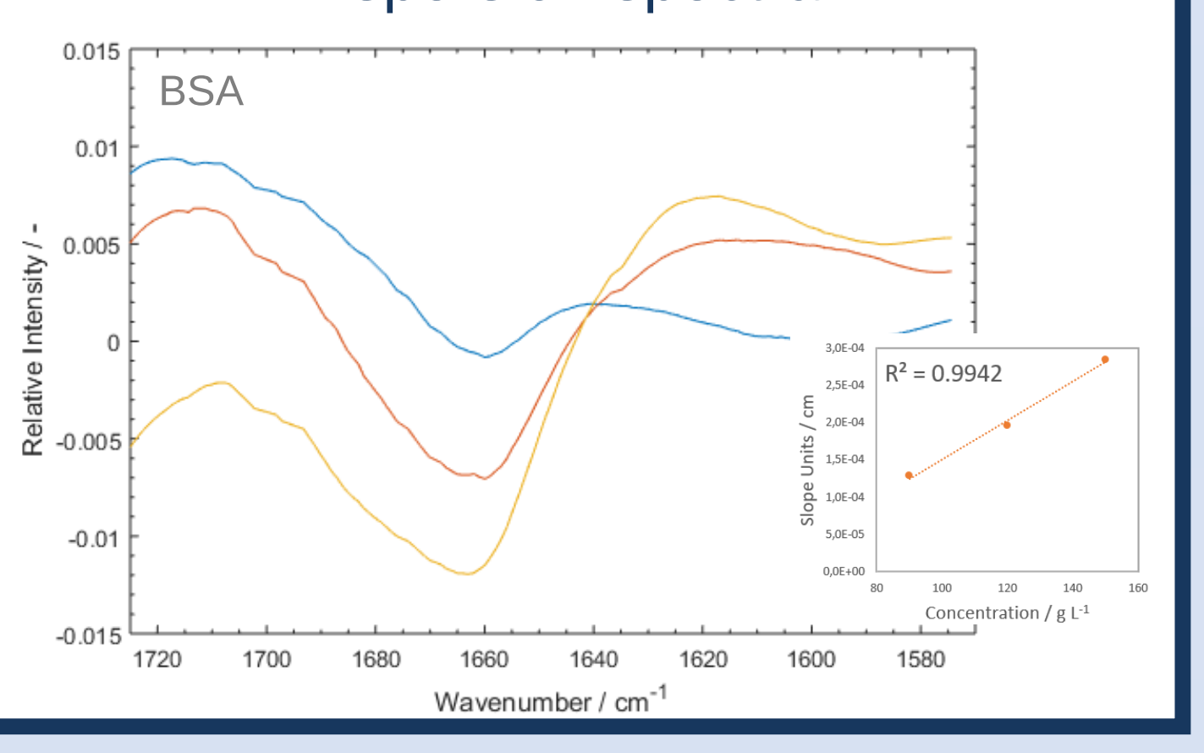
- Quantitative analysis of the dispersion spectra recorded by ATR-MZI setup was performed by assessing the tangent of the signal slope - regions of the rapid change in the refractive index caused by absorption.
- The calibration method confirmed the expected linearity with concentration for the absorption, as well as the dispersion measurements.

Absorption spectra



- For the investigated proteins, limits of detection of 50 mg mL^{-1} (BSA) and 75 mg mL^{-1} (βLG) were achieved.
- Relatively high concentrations required for the setup are due to low interaction path provided by the single-bounce ATR element used in the setup.

Dispersion spectra



Conclusions & Outlook

- The developed experimental setup based on ATR technique successfully proved its capabilities of dispersion spectra acquisition in the amide I band spectral region for the purpose of protein qualitative and quantitative analysis.
- Implemented experimental advancements (i.e. temperature, environmental and sample control) optimized the performance of the developed setup for measurements in the challenging protein spectral region.
- Future outlook: (i) increase of penetration depth to increase the signal and hence the sensitivity of the ATR method, (ii) increase the speed of spectra acquisition, (iii) miniaturization of the setup

References

[1] S. Lindner, J. Hayden, A. Schwaighofer, T. Wolflehner, C. Kristament, M. González-Cabrera, Stefan Zlabinger, and B.Lendl, "External Cavity Quantum Cascade Laser based Mid-Infrared EC-QCL based Mid-Infrared Dispersion Spectroscopy for Qualitative and Quantitative Analysis of Liquid-Phase Samples," Applied Spectroscopy, November 2019

[2] J. Hayden, S. Hugger, F. Fuchs, and B. Lendl, "A quantum cascade laser-based Mach–Zehnder interferometer for chemical sensing employing molecular absorption and dispersion," Appl. Phys. B, vol. 124, no. 2, p. 29, Feb. 2018