# External cavity-quantum cascade laser IR spectroscopy for monitoring temperature-induced α-to-β transition of poly-L-lysine in deuterated solution

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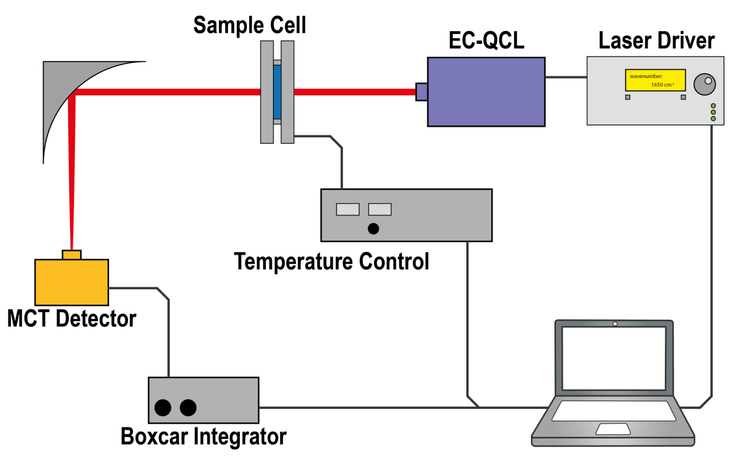
## Introduction

Infrared absorption spectroscopy is a powerful analysis tool to study the secondary structure of proteins. The most prominent absorption band of proteins in the mid-IR spectrum is the amide I band (1600-1700 $cm^{-1}$) which is induced by vibrations of the peptide group. Recently, we have introduced a setup based on a tunable external cavity-quantum cascade laser (EC-QCL) for mid-IR transmission measurements at high optical paths of the protein amide I band in aqueous solution, despite the strong water absorption in this spectral region.

IR spectra of proteins are alternatively acquired in $D_2O$ solution to avoid the overlap between the HOH-bending band of water and the protein amide I band. The DOD-bending band is located at ~1200 $cm^{-1}$, thus creating a region of relatively low absorbance between 1500 and 1800 $cm^{-1}$. In this solvent the amide I band, that is mainly composed by C=O vibrations and to a smaller part by N-H bending vibrations, is shifted to lower wavenumbers (5-10 $cm^{-1}$) due to deuterium exchange.

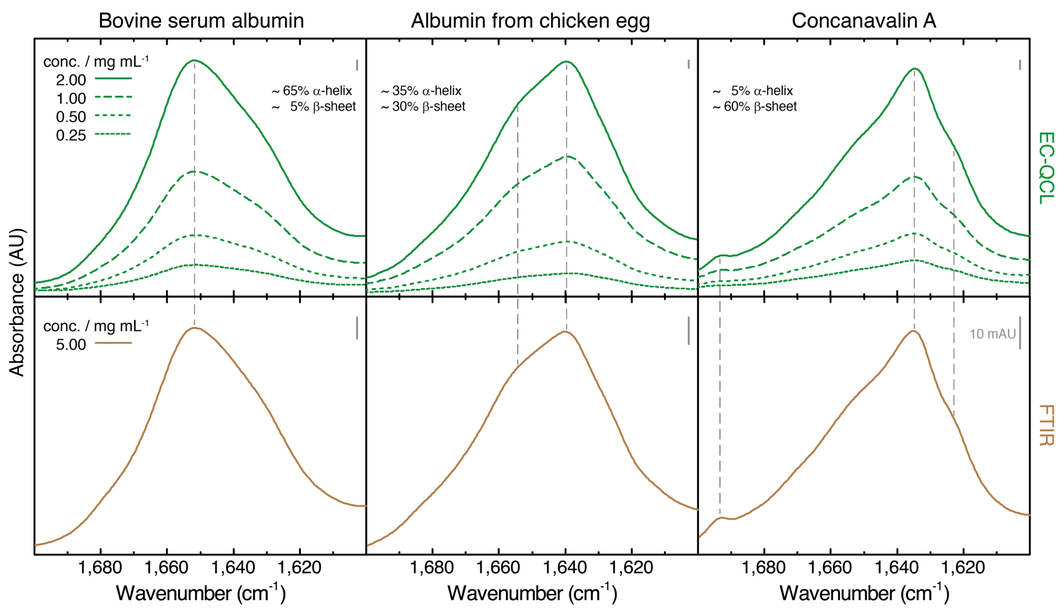
In this work, we apply the EC-QCL-based IR transmission setup for secondary structure analysis of proteins and polypeptides in deuterated solutions at concentrations as low as 0.25 mg $mL^{-1}$. Dynamic IR spectra acquired with the laser-based setup show excellent comparability with spectra obtained by conventional FTIR spectroscopy. By example of the concentration-dependent temperature-induced α-β transition of poly-L-lysine (PLL), we demonstrate the low accessible concentration range for the EC-QCL setup.

## Experimental Setup



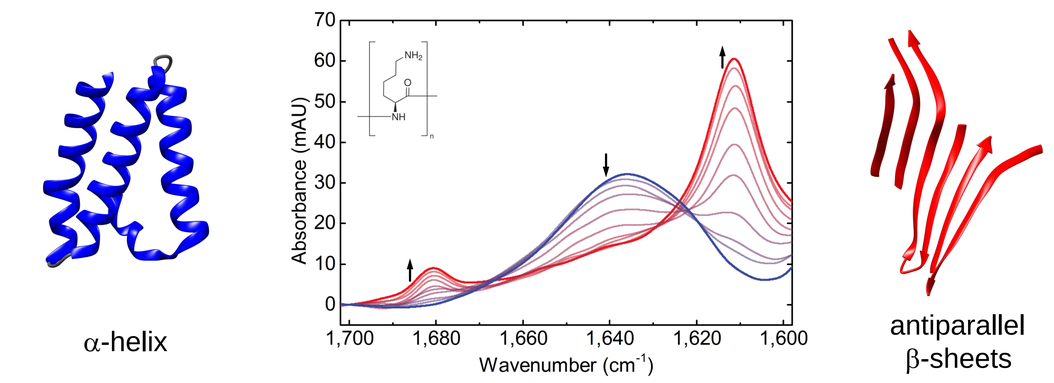

- Laser-IR transmission measurements were performed in pulsed mode using an external-cavity quantum cascade laser (Daylight Solutions, USA) with a tuning range between 1730–1565 $cm^{-1}$.
- Custom-made temperature-stabilized sample cell enabled IR transmission measurements in deuterated solutions at a path length of 478 µm.
- Advanced data processing routine was developed in MatLab employing a Correlation Optimized Warping (COW)-based routine. Inherent mode-hop structures of the laser emission curve are utilized for a rubberband type alignment of consecutive single beam scans. Scan-to-scan alignment allows for scan averaging and background-to-sample alignment reduces the noise level in the absorbance spectrum.
- Wavenumber calibration is performed by referencing the position of the water vapour absorption bands to band positions taken from a spectra database. This type of wavenumber calibration accounts for aberrations originating in the light source and during data acquisition.

## Protein Secondary Structure

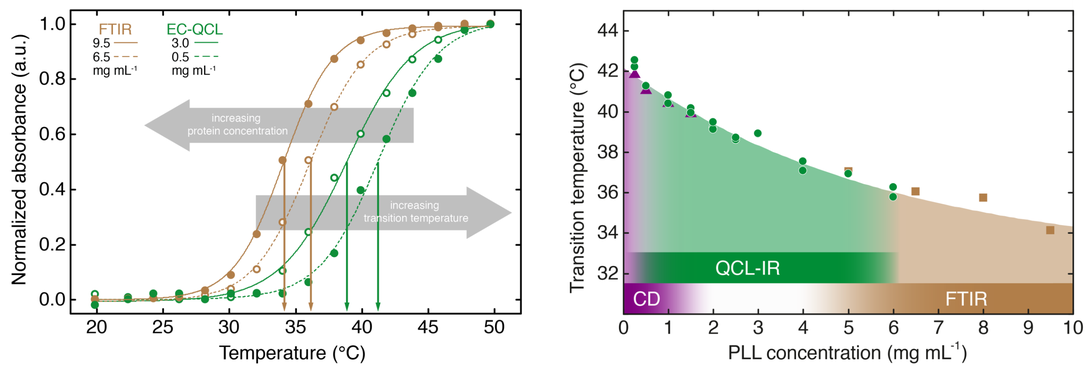



- QCL-based IR transmission measurements were successfully employed to identify characteristic spectral features of proteins with different secondary structures at protein concentrations as low as 0.25 mg $mL^{-1}$.
- Protein spectra acquired by EC-QCL transmission measurements show excellent agreement with absorbance spectra recorded by FTIR spectroscopy.

## Temperature-induced α-to-β transition of PLL




- At pH values higher than PLL pKa (10.5), the lysine side chain is deprotonated and PLL adopts an α-helical conformation. While increasing the temperature up to 50 °C, the structure of PLL transforms into β-sheets.
- The thermally-induced transition of PLL from α-helix to β-sheet involves an intermediate conformation attributed to extended helices or random coil conformation. More intense hydrophobic interactions are the basis for the increased tendency to β-sheet formation at elevated temperatures.
- Temperature-induced conformational changes of PLL were evaluated by monitoring the progression of the IR absorbance at 1611 $cm^{-1}$, characteristic for intermolecular, antiparallel β-sheets. Inflection points of the α-β transition curves were obtained by fitting the data points to a sigmoidal Boltzmann function.
- Employing QCL-IR, FTIR and CD spectroscopy, a concentration dependence of the transition temperature between 0.25 and 10 mg $mL^{-1}$ was revealed which can be explained by the intermolecular nature of β-sheet formation of PLL.




## Conclusions & Outlook

- EC-QCL IR transmission spectroscopy was successfully employed for monitoring temperature-induced conformational changes of PLL in deuterated solution.
- The concentration dependence of the transition temperature between 0.25 and 10 mg $mL^{-1}$ was investigated by QCL-IR, FTIR and CD spectroscopy.
- QCL-IR measurements at biomolecule concentrations as low as 0.25 mg $mL^{-1}$ allow the combination of CD and IR spectroscopy, which is beneficial for comprehensive understanding of complex biological samples.

## References

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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).