

## ImPACts

### Industrial Methods for Process Analytical Chemistry - From Measurement Technologies to Information Systems

Programme: COMET – Competence Centers for Excellent Technologies

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## Proteins in the Focus of Laser Spectroscopy

When working with proteins, an important factor is the overall amount of sample needed for an analysis. Scientists from the TU Wien established a new measurement setup for the analysis of protein structure that requires lower analyte concentrations as compared to the traditionally employed analysis method based on IR spectroscopy. The improvement in performance and robustness has been achieved by employing a novel type of MIR laser light source. This new measurement setup with its extended analysis potential leads the way to new cooperation opportunities within and beyond the PAC research network.



### Folding does matter – Protein structure defines function

Proteins execute an enormous diversity of tasks in living organisms – in plants, animals and in humans. They play an important role in our everyday lives as they contract our muscles, digest the food we eat and transport oxygen in our blood. Proteins can only fulfil their function properly when their three-dimensional structure is arranged in the correct way. Scientists call this process by which proteins assume their functional shape protein folding. If this process is disturbed and proteins adopt a deviating three-dimensional structure, they might not only lose their intended function, but this can also lead to modified or toxic functionality. Most prominent examples for diseases whose origin can be attributed to defective protein folding are the Alzheimer's and Parkinson's disease as well as the mad cow disease (Bovine spongiform encephalopathy, BSE). Also many allergies can be traced back to protein misfolding. Despite the enormous relevance of protein folding, the structure is presently known for only a small

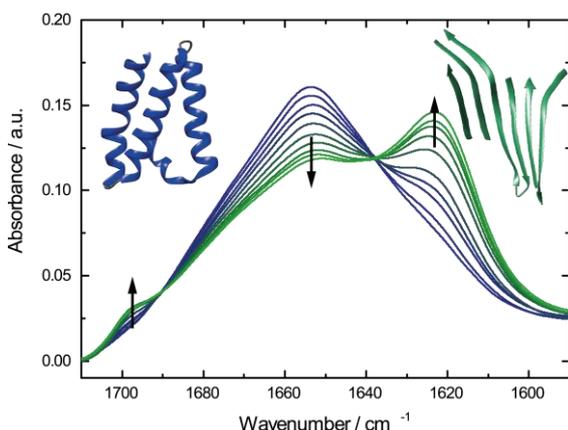
fraction of proteins. One analytical method that is used for analysis of protein structure and its changes is infrared spectroscopy.



### Analysis of Protein structure

Infrared (IR) absorption spectroscopy utilizes the interaction of light in the IR region with vibrations of molecules to obtain information about their chemical structure. Proteins show characteristic absorption bands in the mid-IR region that are specific for individual types of protein structure. In conventionally used Fourier-transform infrared (FTIR) spectrometers broad-band but low intensity light sources are used. Consequently, the intensity of the protein absorbance is rather low. Using higher protein concentrations for analysis would give a more intense signal in the spectrometer. This approach, however, is not feasible in many cases, since production and isolation of proteins at high concentration is hard to achieve. A way to achieve higher signal intensity at low protein concentrations is to employ a light

source with higher output power. Researchers within the PAC network took this approach and employed novel quantum cascade lasers to elaborate a measurement setup for the investigation of protein structure. This new high-output power light source for the IR region has only become commercially available recently. A sophisticated data processing routine has been elaborated to already take full advantage of the potential presented by this new technology. The high intensity of the laser source allows a tremendous increase in ruggedness of the measurements and opens the way to new techniques for investigations of proteins by IR spectroscopy, such as flow injection analysis. This method can be used to study the interaction of proteins with cofactors and ligands. To ensure the acquisition of high-quality spectra, a temperature-stabilization unit for the flow cell has been designed which allows for temperature stability in the range of five thousandth degrees. In a first step, this setup has been already successfully employed for the analysis of protein structure.



**Characteristic change of infrared absorption pattern upon alteration of protein structure.**

## Impact and effects

The advent of microcomputers in the 1970 facilitated the wide propagation of FTIR spectroscopy as a versatile and feasible analytical technique for both scientific and industrial use through a dramatic reduction of measurement time. Since then, several restrictions persisted until now regarding the analysis of aqueous solutions (e.g. blood, electrolytes) due to the inherent characteristics of the ubiquitous solvent of biological samples: water.

Application of new laser light sources with higher output power may be the enabling technology for the employment of infrared spectroscopy in a variety of biomedical applications and life sciences as well as in process analysis. Those new possibilities of measurement shall lead the way to a deeper understanding of mechanisms of protein structure, folding and misfolding and to understanding and perhaps diagnosis of related diseases.

During the last decade, Prof. Bernhard Lendl and his research group at the Vienna Technical University has been playing a leading role in examining the potential of laser light sources for use in infrared spectroscopy. In addition to establishing fundamental knowledge of this new technology, prototypes have been presented for the monitoring of multiple clinically relevant parameters in human blood plasma.

The present work highlighting the analysis of structural properties of proteins has been successfully published in the highly recognized scientific journal "Analytical Chemistry" ([link](#)) and has been recently awarded a poster prize at the international research conference ICAVS 8 (International Conference on Advanced Vibrational Spectroscopy).

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