Infrared Spectroscopy: a TNA offer

V. Raussens, J. Waeytens, E. Goormaghtigh

Center for Structural Biology and Bioinformatics, Laboratory for Structure and Function of Biological Membranes,
Faculty of Sciences, Université Libre de Bruxelles, Brussels, Belgium

Our laboratory and its available equipment, more than 4 FTIR spectrometers with various accessories and highly sensitive MCT detectors, one FTIR microscope with a 128x128 FPA detector, offers **a complete infrared analysis solution to our TNA visitors**. We developed infrared expertise in the analysis of biomolecules over decades now, both in the recording and the interpretation of spectra. We have developed our own analysis program.

The interest of infrared spectroscopy in the study of biomolecules and especially proteins is numerous. Fourier transform infrared (FTIR) spectroscopy is **fast** (a few minutes), required minute amounts of samples (**10-100 ng**) and study proteins **without any external labeling or chemical modifications**.

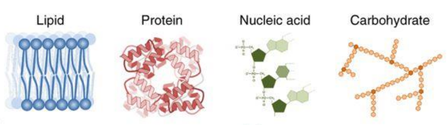
Our laboratory is using mainly the attenuated total reflection (ATR) sampling method which allow an easy study of **membrane proteins in their native lipidic environment** but also poorly or **insoluble proteins like amyloids**.

Information about post-translational modifications like glycosylation or phosphorylation can be acquired also at the same time. In the case of membrane proteins, linear dichroism allows the determination of orientation of the proteins and the lipids. For amyloids, we can distinguish oligomers from fibrils and to follow aggregation kinetics.

We also developed a new **high throughput method** for FTIR, combining an infrared microscope and an array jet protein printer. This was developed in the framework of the Robotein® platform in collaboration with Prof. A. Matagne at the University of Liège (Belgium).

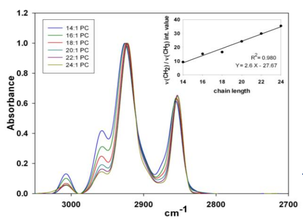
Finally, we recently receive an **AQS³PRO spectrometer** from RedShiftBio. We are now the European reference laboratory for this new technology. This spectrometer can record high quality spectrum in aqueous solution even in the presence of highly concentrated buffer. These **new methodologies are now available** and **included in the TNA offer** provided by the laboratory.

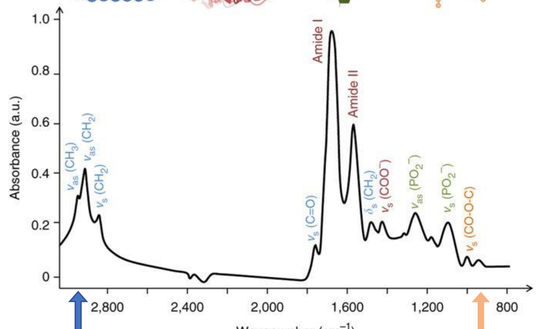
FTIR spectrum and analysis of biological samples



Lipids

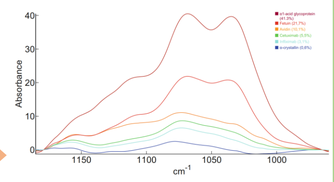
FTIR can give information about the acyl chain length and insaturation in lipids





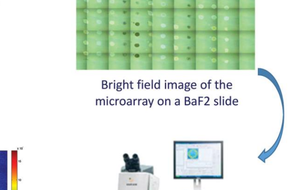
Carbohydrates

FTIR allows the quantification of the glycosylation of a protein



Proteins

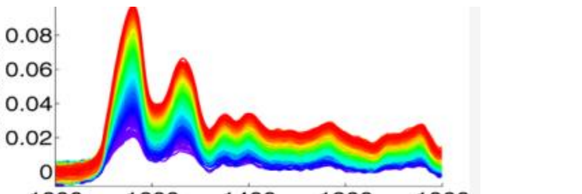
Curve fitting analysis



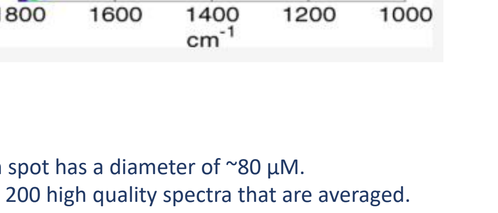
	Amide I 1700 cm^{-1} – 1600 cm^{-1} (ῡ in cm^{-1})	Amide II 1480 cm^{-1} – 1575 cm^{-1} (ῡ in cm^{-1})
α helix	1648 – 1657	1520 – 1543
Unordered Structures	1642 – 1675	1520 – 1543
Parallel β sheet	1623 – 1641	1530 – 1550
Antiparallel β sheet	1623 – 1641 and 1674 – 1695	1510 – 1530

Classically protein structure are obtained by curve fitting analysis knowing the spectral regions of the different secondary structures in amide I

Ascending stepwise linear regression (ASLR) analysis



Sum of absorbance at different wavenumber to obtain 2° structure (see poster #55)



We recorded a spectral database of 92 well-resolved proteins. Using ASLR, we improved the secondary structure prediction.

Attenuated total reflection (ATR) and FTIR analysis

With the ATR mode, there's the formation of an evanescent wave at the surface of the internal reflection element (IRE) interacting with the sample.

ATR-FTIR have multiple advantages:
- No water subtraction needed
- Membrane proteins in their lipidic environment can be studied including their respective orientations.
- No problems with insoluble proteins (e.g. amyloids)
- Gives information on the accessibility to solvant (H/D exchange)

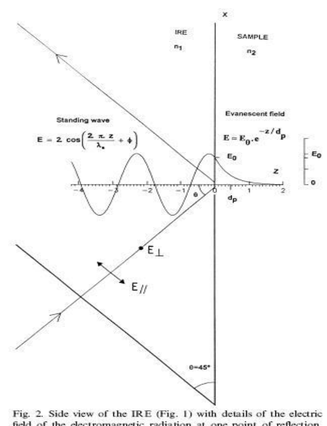


Fig. 2. Side view of the IRE (Fig. 1) with details of the electric field of the electromagnetic radiation at one point of reflection. A standing wave exists within the IRE while the evanescent field decays exponentially outside the IRE. Z in µm.

Linear dichroism and orientation

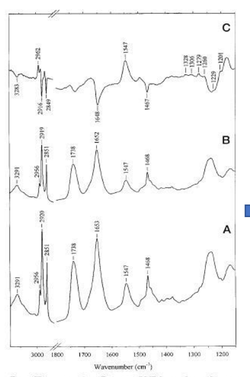


Fig. 2. IR spectra of apoE3(1–183)-DMPC complexes. Spectrum A was obtained with parallel polarized light and spectrum B with perpendicular polarized light. Spectrum C is the dichroic spectrum obtained by subtracting spectrum B from spectrum A. The optical density amplitude of spectrum C has been increased five times with respect to spectra A and B. Measurements were made at 30 °C.

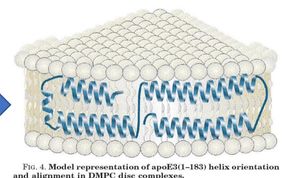
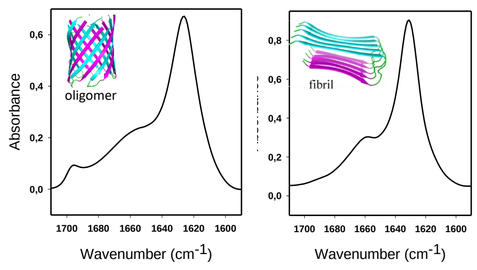


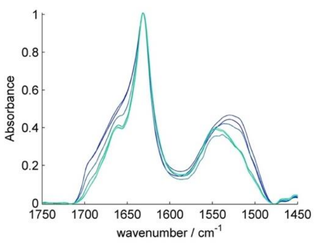
Fig. 4. Model representation of apoE3(1–183) helix orientation and alignment in DMPC disc complexes.

Information on respective orientations allows the design of models of interactions between lipids and membrane proteins

Amyloids



ATR-FTIR Spectra of Ab42 oligomers (left) and fibrils (right)



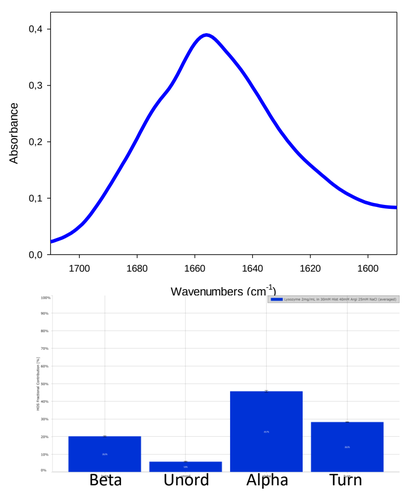
Kinetics of aggregation of Aβ42-Osaka (Δ22) from 0h (dark blue) to 168h (light blue).

Robotein® www.robotein.uliege.be

Robotein® offers automated biophysical protein analysis, together with screening for optimal cloning and gene expression in both bacteria and yeast, and protein purification.

Protein microarrays for high throughput FTIR analysis

Each spot contains 1 ng of proteins (in 100 pL). Each spot has a diameter of ~80 µM.
Each protein is printed in four replicates and gives > 200 high quality spectra that are averaged.



IR spectrum of lysozyme (2 mg/ml) in a 30 mM histidine, 40 mM Arginine, and 25 mM NaCl buffer. Below: results of the automated curve fitting analysis

REDSHIFTbio™ See change

AQS³PRO

"Microfluidic Modulation Spectroscopy, or MMS, enables direct probing of the biophysical structure of proteins. Aggregation, Quantitation, Structure, Stability, and Similarity in a single automated analysis, replacing the requirement to run samples on multiple instruments.
Measurements can be easily performed with high sensitivity to "see change™" over a wide concentration range, from 0.1 mg/mL to over 200 mg/mL without dilution."