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High Pressure Spectroscopic and Microscopic Techniques for Studying Biomolecular Systems

Information obtained from spectroscopies (absorption and emission) can help in revealing structural and dynamic properties as well as the function of biological macromolecules, including processes such as protein folding, aggregation and lipid phase transitions. This module focusses on the application of steady-state fluorescence spectroscopy, Fourier-transform infrared (FT-IR) spectroscopy as well as fluorescence microscopy to study biomacromolecules under high-pressure conditions. Using pressure as physical variable, complementary information on thermodynamic, structural and dynamic properties of lipids, proteins and nucleic acids can be obtained. By means of fluorescence spectroscopy, we will show how the pressure-induced liquid-to-gel phase transition of lipid systems can be detected using the fluorescent probe Laurdan, which is sensitive to hydration and packing changes of the lipid bilayer. Pressure-induced protein unfolding can be monitored using FT-IR spectroscopy up to the multi-kbar regime. In particular, the amide-I' band in the region $1700\text{-}1600\text{ cm}^{-1}$ allows to monitor changes in the secondary structure of proteins. Finally, we will demonstrate instrumentation capable of carrying out fluorescence microscopy studies up to kbar pressures. As an example, measurements on protein condensates will be presented.

In the module offered, we will give a short introduction into the experimental techniques. Practical demonstrations on how to perform experiments, from sample preparation to data acquisition and analysis, will then be given.