Purpose of the visit

The aim of the visit was to join my expertise in the sample preparation and single-molecule fluorescence measurements at ambient temperature with the knowledge and instrumentation of the host in cryogenic experiments, and try to obtain sequences of fluorescence spectra of individual bacterial light harvesting complex of type 2 (LH2). The purpose of this measurement is at least two-fold:

1. single-molecule fluorescence spectrum at low temperature is of interest since the ensemble-associated line-broadening is circumvented; the low temperature insures a simpler scheme of the energy levels contributing to the fluorescence signal – the thermal energy is not sufficient to populate the higher states; therefore such a measurement gives access to the electronic properties of the complex in a rather direct way.
2. spectral time traces yield information about the spectral diffusion; it should be different compared to a situation at ambient conditions since a complex has no thermal energy to undergo spontaneous conformational changes. Therefore the observable spectral changes would be exclusively light-induced. It would be interesting to compare in that regard the low-temperature measurement with the one at ambient temperatures.

Description of the work

1. The first few days I devoted to getting accustomed to the experimental setup and setting the measurement at room temperature – it is a wide-field microscope apposed to the confocal to which I am used. We also had to solve the problem of the stability of the sample since it is very sensitive to the presence of oxygen and in the wide-field configuration not even images of the sample can be obtained unless the oxygen is removed to a certain extent. We managed to reduce the oxygen concentration sufficiently to obtain images and set the right concentration of the sample for the single-molecule measurements.
2. Then we attempted the measurement at liquid nitrogen temperature. We wished to use a different sample immobilization method than the commonly accepted spin-coating in the PVA matrix since we suspect that the latter is damaging for the sample. Therefore we tried to immobilize the sample on a PLL-treated coverslip, submerge it in the physiological buffer and freeze it. This was not successful, however, since the background caused by the fine structure of the formed ice/snow drowned the signal coming from the molecules of interest.

As a simpler and probably less rigorous alternative we tried to immobilize the sample and put it into vacuum, which caused evaporation of the submerging buffer. With this we could observe the single particles although the background was still unacceptably high and originated from the sediment of the evaporated buffer.

Description of the main results

We established the possibility of an alternative LH2 sample preparation for low-temperature measurements and the detection of single LH2s in the wide-field mode. However, for our final goal of monitoring spectral dynamics the problem of the background originating from the immobilization medium has to be solved first.