Report for the short visit Ref 1326

In June 2006, Jérémie Léonard, from IPCMS Strasbourg, visited for 5 days the group of Prof. Majed Chergui at EPFL, Lausanne from the 19th to the 23d. The aim of he visit was to perform a new series of ultrafast spectroscopic measruments on bacteriorhodopsin (bR) proteins and mutants. As compared to previous measurements performed by S. Schenkl et. al. in the group of Prof. M. Chergui with the same set-up, the goal was two-fold: 1) improve the temporal resolution in order to unravel the molecular dynamics in the first 150-200 fs, and 2) compare the dynamics of the wild-type bR and its mutant w182f to the one of the w86f mutants which had never been studied in that way. During previous visists, the experimental set-up, based on two NOPA's (Non collinear Parametric Amplifier delivering intense, visible ultrashort laser pulses), as well as the acquisition procedure had been adapted to these measurements. A preliminary pump-probe signal had been observed by J.L. in a dye in solution (sulforhodamin) as a test experiment.

During this visit, the time resolution of the experiment was first measured by recording the cross-correlation of the pump and probe beams: difference frequency mixing was performed in a thin BBO crystal (50 microns) between the 560-nm pump and 280-nm probe beams. The FWHM of the gaussian-like cross-correlation signal was 80 fs to 100 fs depending on the settings of the NOPA's (short pulse duration requires frequent optimization of the compressors.) The stability of the set-up was also measured: a remarquable pulse-to-pulse stability was observed for the amplified laser system (less than 1 per mille relative noise amplitude at 800 nm) leading to a typical 2% peak-to-peak relative noise amplitude in the UV probe beam. The size at focus and power of both pump and probe beams were carrefully measured 1) to ensure the linearity of the pump-probe signal with respect to the pump intensity and 2) to avoid protein deterioration by too much UV (probe) light intensity. Fresh samples of proteins in solution were prepared by dilution and sonication of concentrated ones. The static absorption spectra were recorded systematically. The optical thinknesss of the samples have to be high for this kind of spectroscopy (OD 5 to 10 per cm, at the pump wavelength), and light scattering by cell membrane fragments or protein agregates is the main source of noise in our experiment. Our attempt in using a detergent to try and reduce this noise was not successfull. However, by averaging during one to two hours for each curve, transient absoprtion signals were recorded successively on the three samples (wild-type bR, w182f, and w86f) with a 550-nm pump wavelength and a 275nm probe wavelength.

Figure 1 shows the transients measured in wild-type bR as well as in the W182F and W86F mutants. These transients are presumably the response of the W182 and w86 tryptophans to the excitation of the retinal molecule nearby. The W182F and wild-type sample had already been compared and show comparable transients. The new result is, that the W86F mutant (where the W86 tryptophan has been replaced by a phenylalanine) shows a signal comparable in intensity to that of the W182F mutant. This was not expected since in wild-type bR, the W86 tryptophan is supposed to be more strongly coupled to the retinal chromophore than the W182 tryptophan. Therefore mutating W86 should lead to a significant change in the transient absorption spectroscopy at this wavelength. These promising and unexpected results as well as the the good laser stability encouraged Jeremie Leonard to come back the week after to continue the measurements.

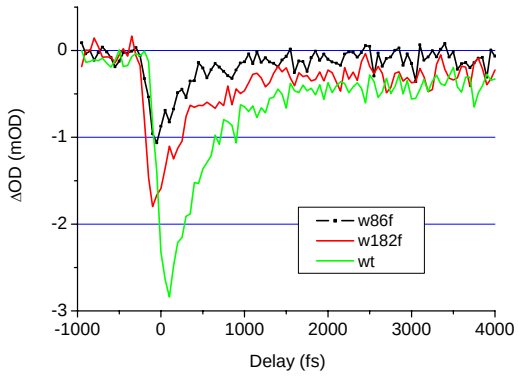

Figure 1 : Transient absorption signal at 275 nm of the wild-type bR and the w86f and W182F mutants.