Effect of point mutations on the ultrafast photo-isomerization of Anabaena Sensory Rhodopsin

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Anabaena sensory rhodopsin (ASR) is a retinal protein that binds the protonated Schiff base of retinal (PSBR) in two ground state conformations: all-*trans*, 15-*anti* (AT) and 13-*cis*, 15-*syn* (13C). Both isomers undergo two distinct photo-cycles that interconvert on a millisecond time scale [1]. The isomer ratio depends on external light conditions (intensity and wavelengths), leading to the hypothesis that the microbial organism uses ASR as a color sensor. In the context of femtosecond spectroscopy and the long-standing question as to how the protein environment controls the photo-isomerization reaction speed and quantum yield (QY), ASR offers the intriguing opportunity to study the isomerization of both PSBRs in the same environment [2]. This is even more relevant, as recent investigations of the light- and dark-adapted proteins by ultrafast transient absorption allowed to single out the dynamics of each isomer [2,3], and showed that the excited state lifetimes (ESL) of both isomers differ by a factor of almost 7 (13C: 110 fs, AT: 750 fs). Notably, while the ground and excited state absorption spectra of both isomers almost overlay, only the different reaction kinetics discriminate 13C and AT. In our extensive study on a series of point mutated proteins, we aim on revealing how the proteins structure controls the isomerization speed and yield of both isomers. Preliminary results on mutants V112N and L83Q enhance the argument for an anti-correlation between excited state lifetime and HOMO-LUMO energy gap, already predicted for rhodopsin proteins by M.Olivucci [4].

Transient absorption experiments were performed with a 1-kHz Ti:Sa amplifier, pumping a home-built NOPA that provides sub-30 fs excitation pulses at 550 nm. A 300-1000 nm white-light continuum is produced in CaF2 and used as a probe. ASR proteins were solubilized in pH 7.0 buffer (150mM NaCl, 50 mM Tris-HCL, 0.02 % DDM). Light-adaptation of all proteins was maintained by constant illumination of the sample reservoir with a 590 nm LED and the isomeric ratio in each sample was characterized by HPLC.

Figure 1A compares the ESA decay kinetics of the DA samples which all have an isomeric content of AT above 90%. The L83Q mutant has the fastest ESA decay (~4 times faster than the WT) while the V112N mutant shows an intermediate behavior. The global fit of each dataset over the 350nm-720nm spectral window reveals the time constants for ESA decay and photoproduct formation. These are 850, 680 and 200 fs for the WT, V112N and L83Q samples respectively, with a typical error bar of 15%.

Figure 1B compares the ESA decay kinetics of the LA species, which consist of a mixture of both isomers with a 13C content of 65% for WT, 50% for V112N and only 36% for L83Q. For the WT case, the same global fit as above yields two ESA decay times of 150 and 640 fs (±15%) with the shortest one attributed to the fast 13C and slower AT respectively, in line with previous works [2,3]. Unlike the WT case, for the V112N mutant, the ESA decay does hardly depend on LA conditions,

and indeed the global fit yields the same ESL of 640 fs (±15%) as for the DA sample. Finally, for the L83Q mutant, the isomeric content of the LA sample remains dominated by the AT. The result from the global fit of the LA sample gave the same 200fs estimate for the ESL.

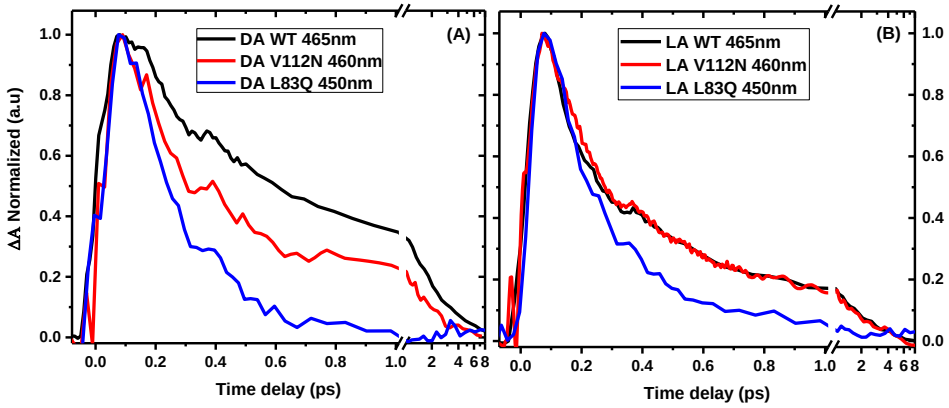

Figure 1: Kinetic traces from the ESA's central probe wavelength of WT ASR and mutants. a) ESA kinetics of the Dark Adapted state (DA) of each sample. b) ESA kinetics for the Light Adapted state (LA) of each sample.

The steady state absorption maxima for the DA samples were located at 550, 530 and 519 nm for WT, V112N and L83Q respectively. Our results show, that an increase of the HOMO-LUMO gap ($\Delta E_{S1\rightarrow S0}$) is simultaneously accompanied by a decrease of the ESL. This seems to enhance the argument for anti-correlation between the two [4].

A precise analysis of how the point mutations change the interactions of the protein environment with the PSBR dipole moments in the ground and excited states will be presented, and how the excited state potential energy surfaces is affected so as to rationalize the effect in the reaction kinetics. At this point modeling of the ESL's of a series of mutants is expected to support the experimental results and by this way will provide new insights to the ultrafast photophysics of retinal proteins and other proteins with photo-switchable isomers.

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