

Ultrafast Excited State Dynamics of the Archae-Rhodopsin 3 and its mutants

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Archaerhodopsin-3 (AR-3), is a VIS light-sensitive retinal protein found in *Halorubrum sodomense*, where it contributes to a primitive form of photosynthesis (transmembrane proton pumping). As most microbial rhodopsins, AR-3 displays a sub-picosecond trans/cis photoisomerization of the retinal chromophore, hence these proteins are non-fluorescent. However, in the early 2010, AR-3 has attracted attention for applications in optogenetics [1]. It was shown

that the voltage-sensitive fluorescence is due to sequential 3-photon excitation needed to induce fluorescence from the photo-cycle intermediate Q [2]. However, multiple mutants then emerged [3], with fluorescence quantum yields (FQY) reaching up to 1.2%, upon 1-photon absorption, which is a 100-fold increase with respect to the wild-type protein (wt). In order to understand this exceptionally strong effect of the mutations in detail, we studied the fluorescence decay kinetics for wt as a function of $\text{pH} \leq 6$, since protonation of the counter ion is known to prolong the excited state lifetime of rhodopsins. Other changes in terms of the electrostatic interactions of the protein cavity with retinal are induced in the double mutant DETC and in the quintuple mutant Arch-5 [3]. The fluorescence kinetics (figure 1) are measured with 200 fs time resolution using a broadband up-conversion set-up. We find them to be best described by a sum of 3 decaying exponentials, which represent the heterogeneity of protein environment. The average excited state lifetimes reach high values up to 65 ps (figure 1). For DETC and Arch-5, the results are in agreement with the reported FQY's [3].

A detailed comparison with QM/MM simulations (V. Ledentu, N. Ferré & M. Olivucci) is in progress.

[1] Kralj, J. M., et al., *Nat. Methods* **2011**, *9*, 90–95.

[2] Maclaurin, D., et al., *Proc. Natl. Acad. Sci.* **2013**, *110*, 5939–5944.

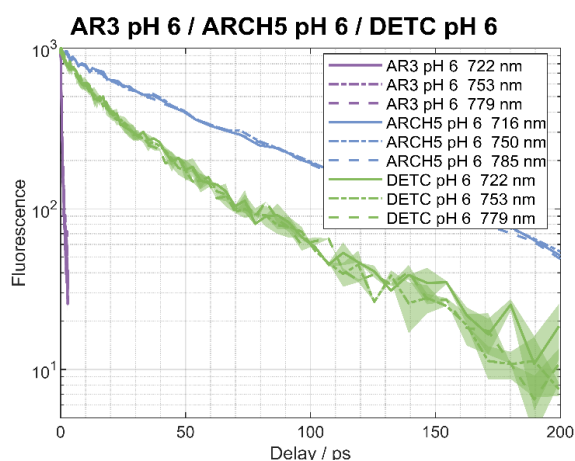


Figure 1: Fluorescence decays of AR-3, and the two mutants DETC and Arch-5, at pH6 on a semi-log scale

[3] Mclsaac, R.S, et al.,*Proc. Natl. Acad. Sci.* **2014**, *111*, 13034-13039.