Vendredi 4 Octobre 2024 à 10h

Attention : jour et heure inhabituelle

Amphithéâtre to be announced, Ecole Polytechnique

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Excited State Dynamics of ArchaeRhodopsin-3 and its Fluorescent Mutants

Archaerhodopsin-3 (AR-3) is a light-driven transmembrane proton pump found in Halorubrum sodomense, that has emerged as an interesting platform for optogenetics [1], since the wild-type form and a large variety of mutants [2] display good fluorescence levels, depending on the cellular transmembrane voltage. Multiple mutants were reported over the years, with fluorescence quantum yields (FQY) reaching up to 1.2%, in the best cases, which is still low as compared to the most fluorescent natural NeoRh [3]. Experimental and theoretical studies aiming at a rational understanding of the mutation-induced ≈100-fold increase of FQY, as compared to wild-type (wt) AR-3, were disclosed recently for the so-called Quasar and Archon families. In the our ongoing project, we focus on the double mutant DETC and the quintuple mutant Arch-5 [2]. A combination of different spectroscopic techniques (fluorescence, transient absorption and Raman scattering), either steady-state or with time resolution down to the 50 fs, allow us to establish that the long-lived fluorescence in the mutant comes from a one-photon excitation process, unlike wt AR-3. Both all-trans/15-anti and 13-cis/15-syn isomers of the protonated Schiff base retinal (PSBR) cation contribute to the fluorescence in the mutants with slightly different excited state lifetimes (ESL). The temperature-dependence of the ESL's allows us to determine the exc. state barrier height, which prevents photo-isomerisation. Indeed, as compared to wt AR-3, the isomerization QY is found to be > 15 times smaller for DETC, and close to zero for Arch-5.

In this talk, I'll discuss more in detail the surprising assessment that similar mutations, aiming at suppressing photo-isomerisation, were not successful in other classes of retinal proteins. Indeed ,it turns out, from quantum chemical simulations performed in the group of M. Olivucci that the electronic properties in the ground and excited states of the PSBR in AR-3 are special. How can this be proven experimentally ?

[1] J. M. Kralj et al, Nat. Methods 9, 2011, 90–95.
[2] R. Scott McIsaac et al PNAS, 2014, 111, 36, 13034-13039.
[3] L. Barneschi et al, Nat. Communication, 2022, 13, 6432.