Thesis project 2024-2027

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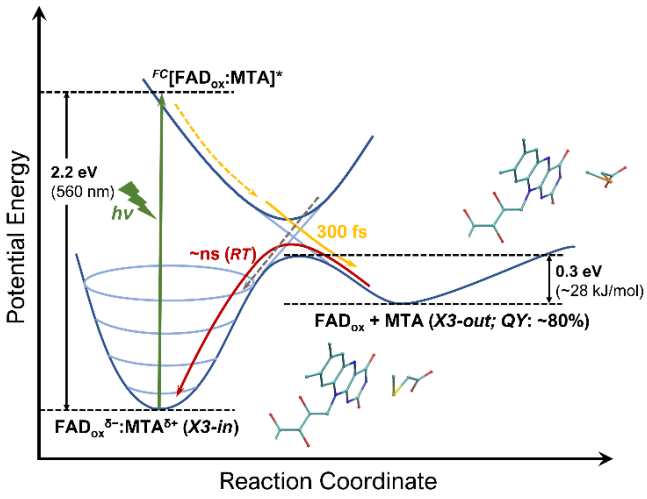
Development of a novel fluorescent photoswitchable protein for nanoscopy

In our group dynamics of light-induced changes in proteins are investigated, including in recent years photocatalytic and potentially photo-transducing processes in flavin (vitamin B2-derivative) containing enzymes [1,2]. The present thesis project is based on the recent discovery in our lab of a novel red photoswitchable system, involving a charge transfer complex formed by the flavin chromophore and a substrate-analogue inhibitor molecule (methylthioacetate, MTA) in the flavoenzyme monomeric sarcosine oxidase (MSOX) [3]. A tentative mechanism was proposed for the reaction (see scheme). This involves motion (proposed to involve MTA isomerization) in the excited state, leading, with high switching quantum yield, in 300 femtoseconds to the photoproduct, and a much slower (nanoseconds), thermally activated, back reaction. This unique novel system provides a promising template for the development of useful red-sensitive biocompatible photoswitches. A collaborative project (ANR PhotoCT, 2023-2027) has just been funded to a) reveal the fundamental mechanism of the reaction in order to b) modify and optimize the system in view of specific applications.



The ANR-funded thesis project will focus on the development of photo-switchable fluorescent MSOX variants for nanoscopy and eventually optogenetic applications. This will be done by removing amino-acid fluorescence quenchers from the flavin environment and by exploring the use of alternative inhibitor molecules in combination with modified substrate cages. The generated complexes will be investigated by time-resolved absorption and fluorescence spectroscopy. The project benefits from in-house biochemical and protein-expression expertise as well as complementary approaches by our external partners who specialize in quantum chemical modeling (Itodys Univ. Paris Cité) and femtosecond crystallography (IBS CEA Grenoble). The candidate ideally has a background in biophysics or physical chemistry with experience in ultrafast spectroscopy and an interest in protein biochemistry.

1. Sorigué, D., Hadjidemetriou, K., Blangy, S., Gotthard, G., Bonvalet, A., Coquelle, N., et al. (2021). Mechanism and dynamics of fatty acid photodecarboxylase. *Science, 372*, eabd5687.
2. Zhuang, B., Ramodiharilafy, R., Liebl, U., Aleksandrov, A., & Vos, M. H. (2022). Ultrafast Photooxidation of Protein-Bound Anionic Flavin Radicals. *Proc. Natl. Acad. Sci. U.S.A., 119*, e2118924119.
3. Zhuang, B., & Vos, M. H. (2022). Photoswitching Behavior of Flavin–Inhibitor Complex in a Nonphotocatalytic Flavoenzyme. *J. Am. Chem. Soc., 144*, 11569-11573