Ultrafast Transient Absorption Spectroscopy of Rhodopsin, Shining Light on Visual Purple Protein

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Rhodopsin proteins play an important role in the vision process. This protein is formed by an opsin associated with chromophore retinal. Under dark, the pigment is present in rhodopsin as 11-cis-retinal; just after illumination, a change in the conformational configuration happens from 11-cis-retinal to all-trans-retinal. This process is the earliest event of the visual process. Significant changes in the geometry of the chromophore are observed, but the time necessary for this change is a few hundred femtoseconds [1].

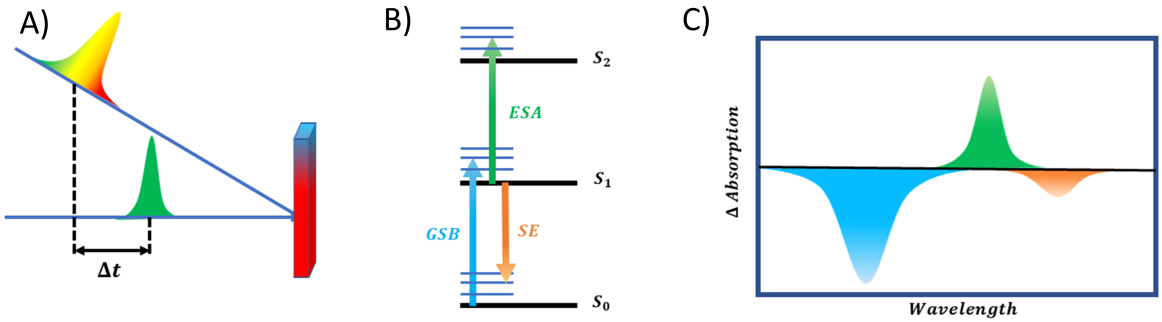

Figure 1: A) Figure 1: A) Schematic description of a Transient Absorption Spectroscopy experimental setup. B) Possible signals obtained in a pump-probe transient absorption experiment. C) Contributions to a transient absorption spectrum: ground state bleach (blue spectrum), stimulated emission (orange spectrum), and excited-state absorption (green spectrum).

In ultrashorts time scales, vibrational coherences play an important role in directing *cis* to *trans* isomerization [2]. Those vibrational coherences can be accessed using ultrafast spectroscopy methods [2,3]. In a typical ultrafast spectroscopy experiment, the third-order optical nonlinearity of the system is monitored. For this, a pump pulse excites the sample to create oscillating dipoles (polarization); a second pulse, delayed by a time interval t_1 changes the sample's population; and a third pulse, delayed by a time t_2 generates the third-order polarization.

Transient Absorption Spectroscopy (TAS) is an ultrafast pump-probe technique that can probe transitions between ground and excited states through ultrafast laser pulses. In a TAS experiment, an ultrashort laser pulse of a few tens of femtoseconds excites the molecules from the ground state to the excited state. After a delay of a few picoseconds, a broadband light pulse probes the system. A TAS spectrum has the main contribution of three processes. A TAS spectrum has the main contribution of three processes. **Ground State Blench** occurs when a decrease in the number of molecules in the ground state as a consequence of promoting a fraction of the molecules to an excited state due to the interaction with the pump pulse leads to a decrease in the absorption signal in the excited sample. As a consequence, a negative absorption is shown in the TA spectrum. **Stimulated Emission** occurs when the pump pulse interacts with the sample and populates the excited state. When a two-level system is considered, the Einstein's coefficients for the ground state absorption (A_{12}) and for stimulated emission from excited state (A_{21}) are identical, and the signal in TAS spectrum will be negative. **Excited state**

absorption takes place when optically allowed transitions from a populated excited state to higher excited states are possible in some wavelengths; the absorption of the probe pulse will occur. The excited state absorption gives a positive signal in the spectrum.

The main goal of this project is to apply the transient absorption spectroscopy technique to understand the ultrafast dynamics undergoing in the rhodopsin protein after excitation. Different types of rhodopsin proteins have been analyzed using TA experiments. Here we propose to perform the experiments in MastR rhodopsin. To our best knowledge, photo excitation dynamics in MastR rhodopsin has not been studied yet.

This project has an interdisciplinary component and is suited for a 3rd or 4th year undergraduate Physics student familiar with and/or interested in Physical-Chemistry, Ultrafast Laser Spectroscopy, and Biophysics. The main tasks for this project are:

- Preparation of protein samples to perform the experiments.
- Perform ultrafast transient absorption spectroscopy on rhodopsin proteins.

The student involved in this project will have the opportunity to have hands-on experience with lasers, the development of skills on how to perform alignment in ultrafast pump-probe setups, and a solid base in ultrafast spectroscopy theory.

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References

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[3] Fishutani, M. Applications of pump-probe spectroscopy Annu. Rep. Prog. Chem., Sect. C: Phys. Chem., 2008,104, 272-297