DIPC Highlight

Understanding Biological Photoreceptors

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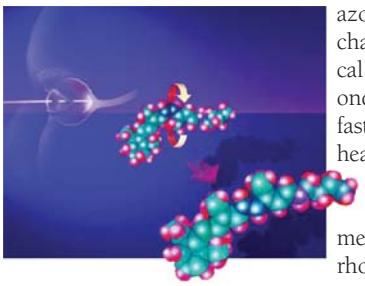
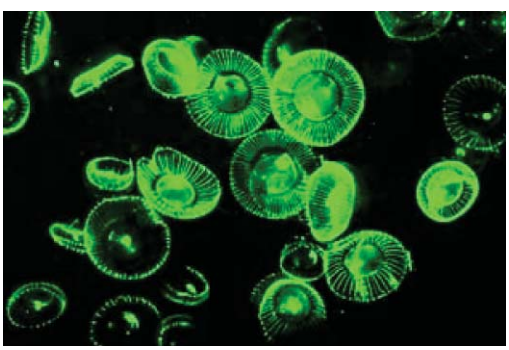
We have implemented a new scheme to describe the photoresponse and structural dynamics of bio-photoreceptors. The scheme can shed light into the initial microscopic origin of vision, photosyntesis and bacteria luminiscence processes.

Chromophore isomerisation is at the heart of biomolecular photophysics.



One of the most fundamental, intriguing and relevant bio-physical process is the microscopic description of the photo-response of bio-molecules. From the photosynthesis to the human vision processes, DNA damage and bacteria bio-luminescence; those phenomena require an understanding of how light is absorbed and how that energy is transfer from the photoreceptor sites to the active biological centers. A famed organic molecule is the azobenzene chromophore, (formed by joining two phenyl rings with the azo group) that undergoes a structural change (cis/trans-isomerization) under optical irradiation, and it does so in a femtosecond time-scale. These light-induced ultra fast rotations around double bonds are at the heart of most photo-active bio-organic reactions. In particular the first step of the human vision are related to cis/trans isomerization of the retinal chromophore of rhodopsin (see figure). The induced structural transformation upon light absorption is very fast (few hundred femtoseconds; 1fs=10^{-15}s) and is associated to the presence of ring structures in the molecule (as benzene or imidazoline groups).

The cited phenomena involve i) different energy (from covalent to van der Waals bonding) and time scales (from fs for the electron dynamics, to ps for the ionic motion and ms for structural reorganizations); ii) light-induced chemical reactions that are intrinsically non-adiabatic, and require techniques that go beyond the traditional Born-Oppenheimer separation of electronic and nuclear degrees of freedom. Therefore, there is a clear need for a reliable theoretical framework to describe processes related to the excited-state dynamics of biological complexes either in vacuo or in solution. In 2002 we joined efforts with the theoretical quantum chemistry group to set up an ambitious project with the goal of developing a theoretical scheme able to predict the photo-induced dynamics in bio-molecular structures. We present here the first results noticing that many other fascinating results are just coming up. Our approach is based on a divide-and-conquer strategy where the active part of the structure is fully described microscopically using quantum mechanics schemes, whereas the environment (i.e., rest of the protein, membrane and solvent) is described using a classical molecular mechanics method (QM/MM approach). The novelty of our work is the simulation of the combined electron-ion dynamics of the photoreceptor within the framework of time-dependent density functional theory (TDDFT). By solving a set of coupled time-dependent Schrödinger-like equations (including light sources as short laser pulses), we identify the mechanisms active at the different time-scales of the photo-process.



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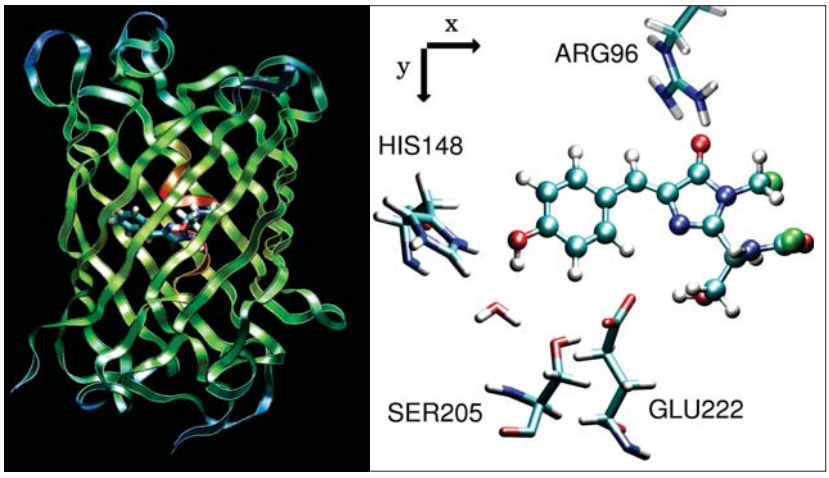

In this context, the Green Fluorescent Protein (GFP) has become a unique tool in molecular biology because of its fluorescent properties and inertness when attached to other proteins. The optical absorption spectrum of the wild type (wt)-GFP, measured at 1.6 K, shows two main resonances at 2.63 and 3.05 eV that are attributed to the two thermodynamically stable protonation states of the chromophore (negative and neutral configurations, respectively). Excitation at either frequency leads to fluorescent green-light emission, peaked at 2.44eV, which is the main mechanism for energy release in wt-GFP. This internal photo-conversion process occurs very rapidly by excited-state proton transfer for the neutral chromophore. The photo-physics of the GFP is governed by a complex equilibrium between the neutral and anionic configurations. Thus, GFP is the ideal system to test our theoretical approach. First we determined the structure of the GFP and then computed the photo-absorption cross section for visible light as it has to be properly described before we could dream of describing any dynamical process.

The GFP protein is folded in a b-sheet barrel conformation with the cromophore occupying a central position inside the barrel (see figure). The cromophore is formed by two consecutive rings, the phenol-type ring of Tyr66 and a five member heterocyle formed by the backbone of Tyr66, the carbonyl carbon of Ser65 and the nitrogen of the backbone of Gly67. The theoretical photo-absorption cross-section is compared to the experiments in the figure (the dashed line corresponds to the neutral chromophore, the dotted line to the anionic, whereas the green and blue curves are the experimental results of S.B. Nielsen et al, PRL87, 228102 (2001) and of T.M.H. Creemers et al, PNAS 97, 2974 (2001), respectively). The strength of the main p - p* transition is larger in the anionic than in the neutral GFP. It is, however, possible to obtain a quantitative description of the spectra of the wt-GFP by assuming a 4:1 ratio for the concentration of the neutral/anionic forms. This value is very close to the estimated experimental ratio of 80% neutral and 20% anionic. Besides the good description of experiments we noticed that GFP is a rather anisotropic molecule in the visible (see inset in the figure). Only light polarized along the pentagon-hexagonal ring is absorbed with high yield. This property can be used to enhance the photo-dynamical processes of GFP samples for opto-electronic devices, e.g. depositing well oriented GFP molecules on top of doped-semiconductor substrates we could get photovoltaic structures with high efficiency.

We have shown that a combined QM/MM and TDDFT approach is able to reproduce the optical response of the GFP. This is a major step toward the first-principles description of excited-state dynamic of important biological photo-receptors. Transient and time-resolved optical spectroscopy could be studied. However, in spite of the good agreement, some questions remain open. For example, how does the excitation in the GFP trigger the proton shuttle mechanism and what is the time-scale for this process? To answer these questions we have to go beyond the present work including the excited state dynamics of the environment (e.g. the proton-transfer involves structural modifications of the environment that have to be properly described). Work along this line is in progress. ■

Our TDDFT approach constitutes a major step towards the first principles description of the combined electron/ion dynamics in bio-photoreceptors.

REFERENCE
M.A.L. Marqués, X. López, D. Varsano, A. Castro and A. Rubio, Physical Review Letters **90**, 258101 (2003)