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Observe while it happens: catching photoactive proteins in the act with non-adiabatic molecular dynamics simulations

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Organisms use photo-receptors to react to light. The first step is usually the absorption of a photon by a prosthetic group embedded inside the photo-receptor, often a conjugated chromophore. The electronic changes in the chromophore induced by photo-absorption can trigger a cascade of structural or chemical transformations that culminate into a response to light. Understanding how these proteins have evolved to mediate their activation process has remained challenging because the required time and spacial resolutions are notoriously difficult to achieve experimentally. Therefore, mechanistic insights into photoreceptor activation have been predominantly obtained with computer simulations. Here we briefly outline the challenges associated with such computations and review the progress made in this field.

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Introduction

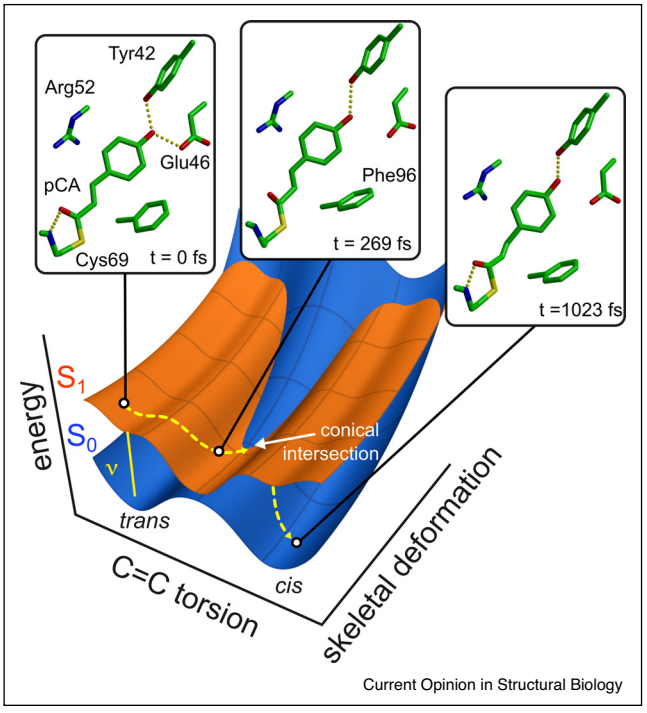
Life on earth is powered by the sun. To harness its power, organisms have developed a wide range of mechanisms for collecting and storing solar energy. In addition, many organisms have also evolved photo-sensory proteins to detect and respond to light. While the various photo-response mechanisms may be highly diverse, they share the common feature that the first step consists of the absorption of a photon by a chromophore co-factor inside the protein matrix. The sudden electronic response of that chromophore to photo-absorption can subsequently trigger a series of chemical or conformational changes that ultimately leads to the capture of the photon's energy into a covalent bond or to the activation of a signaling cascade.

High-resolution structures of photoactive proteins have been obtained with X-ray crystallography and NMR spectroscopy, but these techniques can only capture static snapshots of what is essentially a dynamic process. While the dynamical response to photon absorption can be probed accurately with transient spectroscopy methods, these techniques are only sensitive to molecular energy levels and thus do not provide atomistic information about the photo-induced structural changes. Therefore, experimentally it remains challenging to simultaneously access the time and spacial resolutions required for understanding the activation mechanism of photoactive proteins in atomic detail. In contrast, computationally the relevant time and spacial resolution can both be accessed routinely, and molecular dynamics (MD) computer simulations have become the method of choice to advance our understanding of how these proteins have evolved to mediate their photochemistry. Indeed, since MD trajectories contain information about the time-evolution of both atomic positions and energies, simulations provide a link between static structure determination and transient spectroscopy. Here, we outline the main challenges associated with modeling the ultra-fast dynamics when photoactive proteins absorb light, and illustrate the progress made in this field with recent applications. We conclude this review with an outlook on how we expect this field to develop further.

Challenges

Photon absorption induces a transition from the electronic ground state (S_0) into an electronic state of higher energy, usually the first singlet excited state (S_1). Because the potential energy surface associated with an electronic excited state differs from that of the electronic ground state, photo-absorption can trigger an immediate dynamic response (Figure 1). However, due to the over-damped and highly dissipative molecular surroundings of proteins, part of the energy released during the initial structural relaxation is quickly lost. Furthermore, the total excited state lifetime is limited due to spontaneous emission. Therefore, to make sufficient energy available for the downstream chemistry under these constraints, it is imperative to decay rapidly from the excited state into a metastable product on the electronic ground state that is chemically or structurally different from the initial configuration (Figure 1). Such decay occurs most efficiently

Figure 1



Schematic representation of the potential energy surfaces involved in a photo-chemical reaction. The photo-isomerization in Photoactive Yellow Protein is used as an example [2]: upon photon absorption, the covalently bound *para*-coumaric acid chromophore (pCA) undergoes rapid rotation around the central double bond from the *trans* configuration into a 90° twisted configuration. In this twisted geometry, the electronic ground (S_0) and excited (S_1) states are nearly degenerate, and radiationless deactivation to S_0 occurs. After decay, the system relaxes into a new local minimum on the S_0 potential energy surface, associated with a strained *cis* chromophore that stores sufficient energy for subsequent conformational changes [3].

at or near so-called *conical intersections* between the ground and excited state potential energy surfaces [1]. Thus, modeling photo-induced chemistry in photoactive proteins requires, in addition to a structural model, an accurate description of the excited-state electronic wave function, as well as an approach to deal with the radiationless deactivation at conical intersections.

Excited-State Quantum Chemistry

While quantum chemistry has matured to the point that chemical accuracy can be achieved for processes that occur in the electronic ground state, the possibilities to model excited states are still limited as reliable methods that can provide a balanced description of both ground and excited states, are available only for small molecules and at high computational expense [4]. Owing to their typical size and complexity, modeling the excited states of photo-receptors therefore requires additional approximations, ranging from using isolated molecular cluster models [5] to hybrid quantum mechanics/molecular mechanics (QM/MM) treatments [6], in which the atoms involved in the photo-chemistry are described at a suitable level of *ab initio* theory or time-dependent density functional theory (TDDFT), while the remainder of the system, including the rest of the protein, solvent and ions, is modeled with a computationally cheaper molecular mechanics force field [7].

Non-adiabatic molecular dynamics

At a conical intersection between electronic potential energy surfaces, the adiabatic, or Born-Oppenheimer picture, in which molecular dynamics evolve on a *single* potential energy surface, breaks down [8]. Instead, near the intersection the electronic states are coupled and nuclear motion can induce so called non-adiabatic transitions between these electronic states. Although the break-down of the Born-Oppenheimer approximation implies that also the nuclear dynamics can no longer be described with Newton's equations of motion, the non-adiabatic effects can be satisfactorily modeled with so-called *semi-classical* MD methods [9]. A popular approach to include non-adiabatic transitions in classical MD simulations is Surface Hopping. Introduced by Tully over three decades ago [10], the trajectory can hop between two potential energy surfaces. The hops are instantaneous and triggered by changes in the quantum mechanical populations of the electronic states due to their non-adiabatic coupling with nuclear degrees of freedom [9]. A promising alternative to surface hopping is the full multiple spawning (FMS) method of Martínez and co-workers [11], which solves the electronic and nuclear Schrödinger equations simultaneously and has been adapted to QM/MM simulations [12].

Exciton dynamics in multi-chromophoric systems

In order to capture photons under the low light intensities in some habitats on earth, photosynthetic organisms evolved elaborate strategies for increasing the absorption cross-section of their photosynthesis apparatus. Thus, in addition to reaction centers, in which photons are transformed into chemical free energies through photo-induced redox chemistry, these organisms express light-harvesting proteins that self-organize into large complexes around the reaction centers [13]. Because these proteins contain one or more chromophores, the likelihood of absorbing a photon increases, while the absorbed photon is quickly channeled into the reaction center through the excitonic couplings between the chromophores.

Simulating these processes on a computer would in principle require that *all* chromophores are included inside the QM region. However, since excited state quantum chemistry is challenging even for a *single* chromophore [4], calculating the excited state dynamics of a multi-chromophore system would be computationally prohibitive unless additional approximations to the ones already introduced above, are made. In the exciton model

[14••, 16,17•,18] rather than including *all* chromophores in a single quantum chemistry calculation, the excited state wave functions are computed for each of the chromophores separately instead. The excitonic Hamiltonian, containing the excitation energies of the chromophores on the diagonal and the dipole–dipole excitonic couplings between the chromophores as off-diagonal elements, is diagonalized to yield the multi-chromophore wave functions, energies, gradients and non-adiabatic couplings, required for semi-classical MD simulations of multi-chromophore complexes [15,17•] (Figure 2).

Applications

While the first semi-empirical excited-state MD simulation of retinal in a Rhodopsin model dates back more than four decades [19], the field did not mature until the early 2000s, when crystal structures of several photoreceptors were available and computer technology had advanced enough for fully atomistic QM/MM simulations of Bacteriorhodopsin [20,21] and Photoactive Yellow Protein [2]. The total number of trajectories in those early studies was relatively low, but nevertheless yielded a consistent picture of the photo-isomerization process and the role of the protein environment. Since then, excited state dynamics simulations have been used to investigate retinal photo-isomerization in other proteins as well, including bovine Rhodopsin [22–24], squid Rhodopsin [25], Melanopsin [26], channel Rhodopsin [27•], and Halorhodopsin [28,29]. Furthermore, non-adiabatic QM/MM trajectories have also been computed for chromophore photo-isomerization and excited-state proton transfer in various fluorescent proteins [30,31,32,33•,34].

Comparison to time-resolved spectroscopy

The accuracy of the QM/MM simulation models, which often rely on a minimal QM region embedded within a non-polarizable MM environment, remains a matter of concern. Because experimental data on ultra-fast structural dynamics are still very rare, the validity of the simulations can only be verified *indirectly* by computing and comparing spectroscopic observables. While quantum yields and excited-state lifetimes are relatively straightforward to compute and compare, it is also possible to compute the time-evolution of spectral signals, and compare to data from fluorescence up-conversion or transient absorption experiments [23]. For example, the agreement between the fluorescence decay calculated from 60 FMS trajectories of retinal photo-isomerization in Channelrhodopsin-2 [27•] and experiment [35] suggests that despite the approximations, the QM/MM simulations can capture the non-adiabatic dynamics in this system rather accurately (Figure 3a).

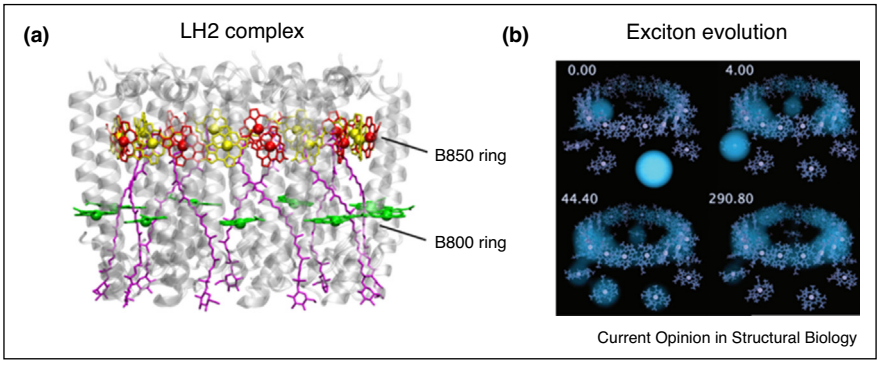
Comparison to two-dimensional electronic spectroscopy

The energy flow inside coupled multi-chromophoric systems, such as light harvesting complexes, is conveniently probed with two-dimensional electronic spectroscopy, a technique that correlates excitation and emission energies as a function of time delay between absorption and emission [37,38]. Thus, the validity of simulations of a multi-chromophore system can verified by computing the 2D electronic spectra. In Figure 3b, the calculated [14••] and measured 2D electronic spectra of Light Harvesting complex II are compared [36]. The good agreement also here suggests that the underlying simulation model is sufficiently accurate and that the simulations can be used to guide the interpretation of the measured 2D signals [14••].

Comparison to time-resolved serial femtosecond X-ray crystallography

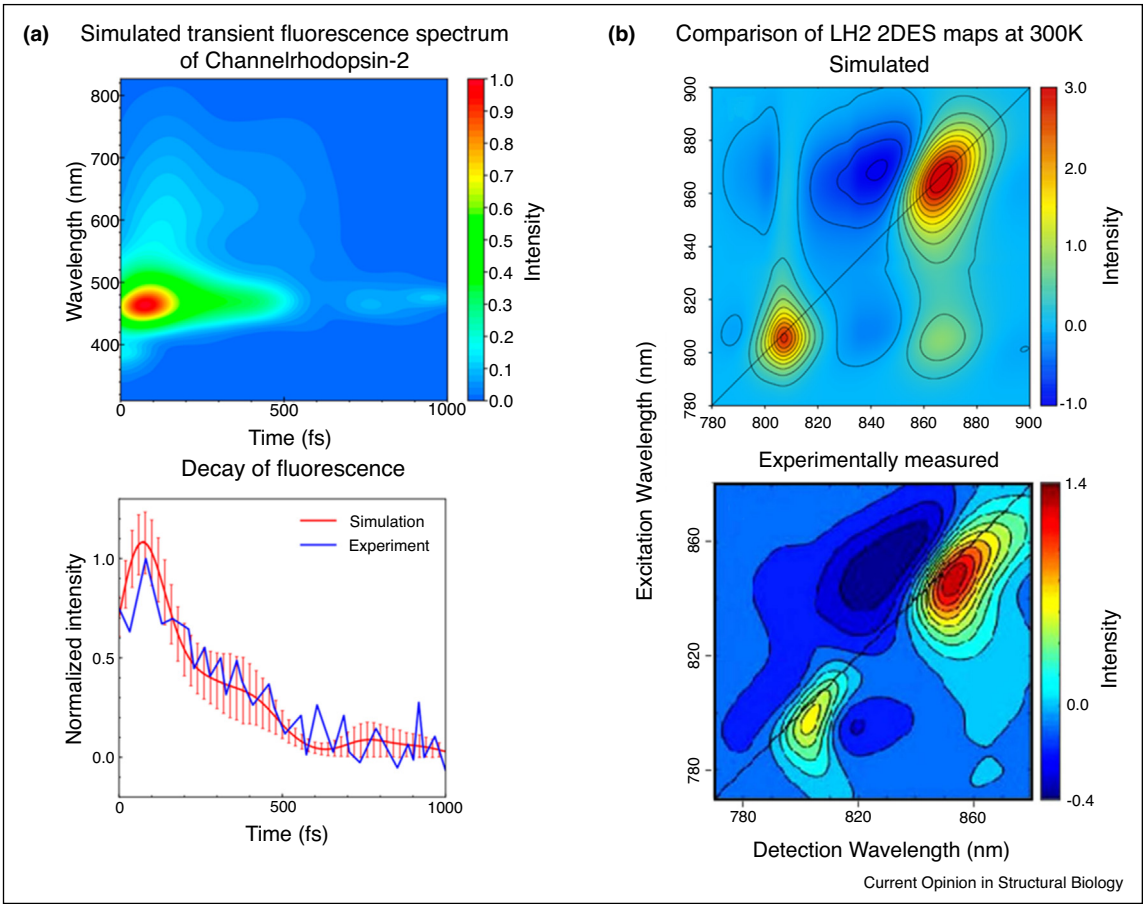
Time-resolved crystallography at free electron lasers has opened up a new experimental window into the regime of ultra-fast molecular dynamics [39]. Thanks to femtosecond pulses of extreme brilliance (i.e. 10^{12} hard X-ray

Figure 2



Panel **(a)**: The light harvesting complex II (LH2) from *Rhodoblastus acidophilus*, containing 27 Bacteriochlorophyll-a chromophores (18 B850, 9 B800) that are excitonically coupled (reprinted with permission from [14••]. Copyright 2017 American Chemical Society). Panel **(b)**: Snapshots of a non-adiabatic MD trajectory that show how the excitation, initially localized on one of the chromophores, evolves through the LH2 complex (reproduced from Ref. [15•] with permission from the PCCP Owner Societies).

Figure 3



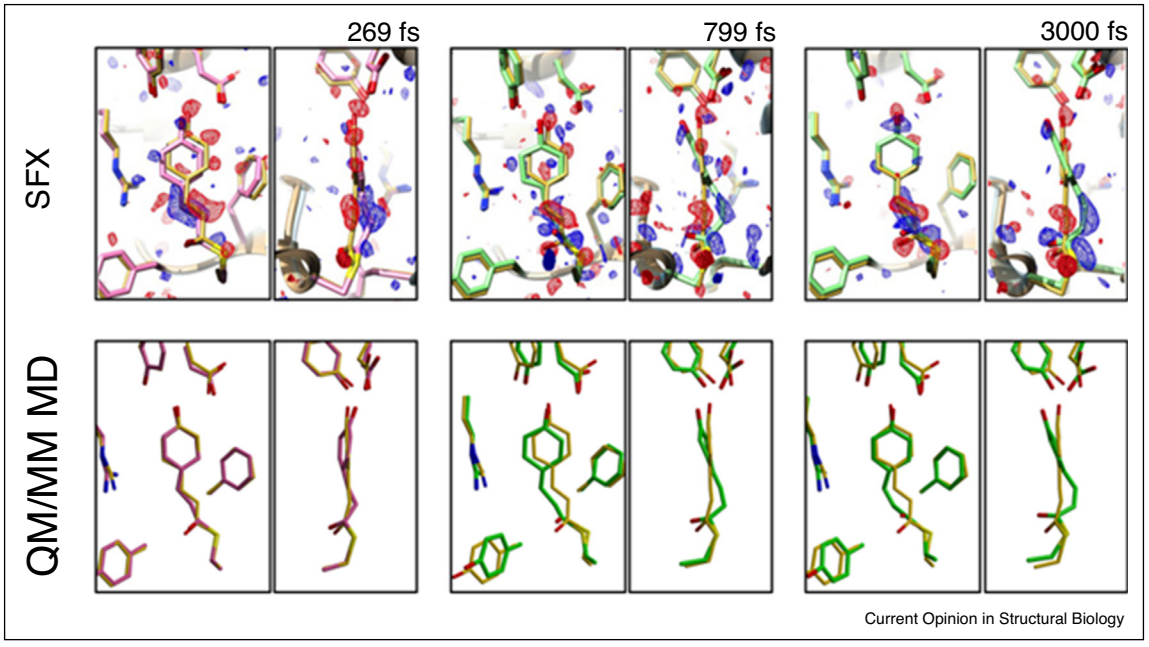
Panel **(a)**: time-resolved emission (top) and fluorescence up-conversion signal at a single wavelength (bottom) of Channelrhodopsin-2 computed from 60 FMS trajectories (red) [27•], compared to experiment (blue) [35] (reprinted with permission from [27•]. Copyright 2019 American Chemical Society). Panel **(b)**: Simulated [14••] and measured [36] two-dimensional electronic spectroscopy pump-probe maps of Light Harvesting complex II (LH2) (reprinted with permission from [14••]. Copyright 2017 American Chemical Society).

photons per pulse [40]) it has become possible to perform pump–probe experiments with X-rays on photoreceptor micro-crystals or nano-crystals. A short optical pump pulse initiates the photo-chemistry, while a femtosecond X-ray pulse probes the diffraction after a time delay [41]. Because the probe pulse captures a single protein crystal in a random orientation, a large series of diffraction patterns needs to be collected for each pump–probe delay, indexed and merged before the changes in electron density due to photon absorption can be calculated.

Recently Schmidt and co-workers have applied this time-resolved Serial Femtosecond X-ray crystallography technique (tr-SFX) to Photoactive Yellow Protein [42]. Figure 4 shows how the electron density difference in the chromophore binding pocket evolves as a function of time after photo-excitation with a 140 fs laser pulse at 450 nm. Comparing the transient structures obtained by refining extrapolated difference structure factor amplitudes, to the structures of a non-adiabatic multi-configurational QM/MM trajectory, suggests a remarkably good agreement between simulation and experiment. Similarly, also for rsEGFP2, a photo-chromic variant of the *Aequorea victoria* Green Fluorescent Protein, agreement was found between semi-empirical QM/MM simulations and tr-SFX experiments [33•].

However, due to the very high power of the optical pump laser, it remains unclear whether these early experiments probed the biologically relevant photo-isomerization mechanism. Indeed, a recent power titration of Bacteriorhodopsin suggest that with the laser powers used in those tr-SFX experiments, the initial dynamics is dominated by multi-photon absorption rather than the biologically relevant single photon process [43••]. This finding not only raises questions about the interpretation of a preceding SFX study on Bacteriorhodopsin [44], but of all femtosecond time-resolved SFX results on photoactive

Figure 4



Comparison between time-resolved crystallography (upper panel) and non-adiabatic MD simulation (bottom panel) of Photoactive Yellow Protein. Each panel shows a front and side view of the chrompohore pocket as a function of pump-probe delay. In the top panels, the experimental electron difference densities with respect to the unactivated resting state of the protein are shown. Red contours indicate loss of electron density whereas blue contours indicate gain. In all panels the X-ray structure of the resting state is shown in yellow, while the structures refined against the extrapolated difference structure factor amplitudes, are shown in pink and green [42].

proteins so far. Although very challenging, it is possible to simulate the dynamics after multi-photon absorption. Therefore, this issue could in principle be addressed with non-adiabatic MD simulations as well.

A further limitation of SFX is that dynamics can only be probed inside crystals. Because crystal packing could restrict the conformational movements of the protein, the structural changes might be different in solution. Indeed, comparing X-ray scattering from phytochrome photoreceptor proteins in solution on the one hand, and X-ray diffraction from these proteins in crystal on the other hand, suggests that the photo-induced structural changes are much larger in solution than in the crystal [45]. However, because large-scale conformational changes typically occur on timescales that are much longer than the excited state lifetime, the effects of crystal packing are probably negligible on such ultra-fast timescales. Nevertheless, also here, MD simulations can be used to systematically investigate the differences between the conformational dynamics in crystals and in solution.

Summary and outlook

Nature has evolved various strategies for harvesting, converting and storing solar energy. Understanding these strategies might be the key to new solar technologies. While tr-SFX at free electron lasers will most certainly lead to new and unprecedented insights into the inner workings of Nature's photoactive protein machinery, this technique is still in its infancy and access to free electron facilities is limited. However, the agreement between simulations and SFX experiments suggest that despite limited accuracy, non-adiabatic molecular dynamics are a good alternative to SFX. Future developments will undoubtedly lead to more accurate QM/MM models with, for example, a multi-reference rather than multi-configurational description of the QM region, as recently demonstrated for an isolated GFP chromophore [46••], or with a polarizable MM environment rather than static point charges [47]. Furthermore, in contrast to experimental data, trajectories can be analyzed at the atomistic level and thus provide insights beyond the reach of experiment. For example, information about the interactions between chromophore and protein environment, which is essential to understand the catalytic effect of the protein, can be obtained much easier from simulations than experiment and hence lead to new testable hypotheses. Because these insights can be crucial also for designing new systems, combining experiment and computation, in particular with a high-throughput strategy [48•], might be a promising avenue towards new

discoveries in photobiology, as well as future technologies for harnessing the power of the sun.

Conflict of interest statement

Nothing declared.

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