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Light-induced Dynamics in Biomolecules: A Challenge for Computer Simulations

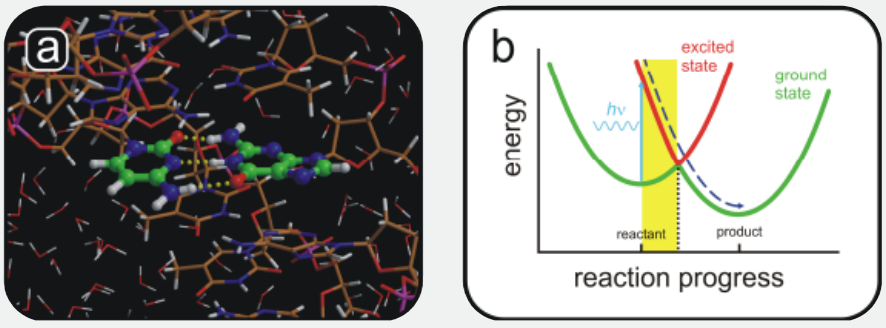
Lars Schäfer, Gerrit Groenhof and Helmut Grubmüller **Department of Theoretical and Computational Biophysics**

Organisms have evolved a wide variety of mechanisms to utilize and respond to sunlight. In many cases, the biological response is mediated by structural changes that follow photon absorption in the biomolecule. Exposure to high-energy photons, however, can cause irreversible damage in biological matter and even alter the genetic information. Despite the relevance of these photochemical processes in biology, little is known about the atomistic mechanisms by which irradiaton can bring about changes in the chemical structure.

Understanding these photobiological reactions requires insight into the underlying ultrafast molecular dynamics, which typically occur at femto- to picosecond timescales (10^{-15}– 10^{-12} s). As the relevant time and spatial resolutions are notoriously hard to access experimentally at atomic resolution, atomistic computer simulations are essential for studying photobiological processes. Here we report on mixed quantum/classical molecular dynamics (MD) simulations (see box) to reveal the detailed sequence of structural changes that follow photon absorption in biomolecules. In this contribution, we present recent applications on (i) activation of a photoreceptor protein and (ii) benign and malign photochemical reactions in DNA.

Molecular Dynamics Simulations of Photobiological Processes

In MD simulations, Newton's equations of motion are solved to obtain a trajectory of the dynamics of a molecule over a period of time, starting from an X-ray or NMR-derived structure. To model the electronic rearrangement upon photon absorption requires a quantum mechanical (QM) description (CASSCF, CASPT2) of those parts of the system that are involved in the reaction. For the remainder, a simple molecular mechanics (MM) forcefield model suffices. Both models are combined in a hybrid QM/MM scheme.



(a) DNA molecule used in our MD simulations. The QM subsystem is shown in ball-and-stick representation; MM atoms are shown as thin sticks. **(b)** To follow the dynamics of a photoactivated reaction in a mixed quantum/classical (QM/MM) simulation, both the electronic ground state (green) and the excited state (red) have to be accurately described. Photon absorption (blue arrow) brings the system to the excited state (yellow shaded area), but after a radiationless decay (dashed line), the system ends up in the ground state. The radiationless transitions between the electronic states are modelled with a surface hopping algorithm.

Photoactivation of a Bacterial Photoreceptor Protein

Photoactive Yellow Protein (PYP) is believed to be the primary photoreceptor for the photo avoidance response of the salt-tolerant bacterium *Halorhodospira halophila*. PYP contains a deprotonated 4-hydroxy-cinnamic acid (or *p*-coumaric acid, pca) chromophore linked covalently

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Herausgeber:
Presse- und Öffentlichkeitsarbeit
<pr@mpibpc.mpg.de>

Max-Planck-Institut für biophysikalische Chemie
Am Fassberg 11
37077 Göttingen
Tel +49 551 201-0
Fax +49 551 201-1222
www.mpibpc.mpg.de

to the γ-sulphur of Cys69 via a thioester bond (green, Figure 1). Upon absorbing a blue-light photon, PYP enters a fully reversible photocycle involving several intermediates on timescales ranging from a few hundred femtoseconds to seconds.[1]

By means of QM/MM MD simulations, we have revealed the detailed sequence of structural changes that follow photon absorption.[2] The crucial step is a light-induced *trans*-to-*cis* isomerization of the covalently bound chromophore (Figure 1). In vacuum, this photoisomerization reaction does not occur, demonstrating that the protein environment is essential for the photoreaction. Furthermore, mutating one of the key amino acids in the chromophore cavity alters the photochemical reaction and leads to a different photoproduct.[3] These observations, which are difficult to predict by other means, demonstrate that the chromophore's photoreactivity is extremely sensitive to the surrounding protein environment.

Figure 1 shows the primary events after photoexcitation of the wild-type protein. The chromophore rapidly decays to the ground state *via* a 90° rotation of the double bond (torsion *b*, Figure 1). Upon returning to the ground state the chromophore relaxes back either to the original *trans* conformation (0°), or it continues isomerizing to a *cis* conformation (180°). In the latter case, the relaxation also involves a flip of the thioester linkage, in which the carbonyl group rotates 180°. During this rotation, the hydrogen bond between the carbonyl oxygen and the Cys69 backbone amino group is broken. In total, 14 MD simulations were carried out, initiated from different snapshots from a ground state trajectory. The excited state lifetime (200 fs) and isomerization quantum yield (30 %) in our simulations agree well with experiments (400 fs and 35 %, respectively). Furthermore, we found that in the protein environment the isomerization is enhanced by a preferential electrostatic stabilization of the chromophore's excited state by the guanidinium group of Arg52, located just above the negatively charged chromophore ring.

PYP mutants in which the arginine has been replaced by an uncharged aminoacid can still enter the photocycle, albeit with a lower rate and quantum yield.[4] These findings indicate that Arg52 is important but not essential for photoactivation. To elucidate the role of this arginine in the activation process in more detail, we have also performed excited state dynamics simulations of the Arg52Gln mutant of PYP. In the mutant, the primary events after photo-excitation are different from those in the wild-type. First, the predominant excited state reaction in the mutant involves isomerization of a *single* bond in the chromophore (torsion *a*, Figure 1), rather than the double bond (torsion *b*). Although single bond isomerization does not result in the formation of the *cis* chromophore, a 180° flip of the thioester group and a rupture of the hydrogen bond to Cys69 was observed with a 20% quantum yield. Together with the experimental observation that the mutant has a photoactivation quantum yield of about 20 %,[4] this suggests that the key step to enter the photocycle is the oxygen flip rather than the double bond isomerization. The second difference is that the photochemical process is considerably slower in the mutant than in the wild-type by a factor of about three, in agreement with recent measurements.[5]

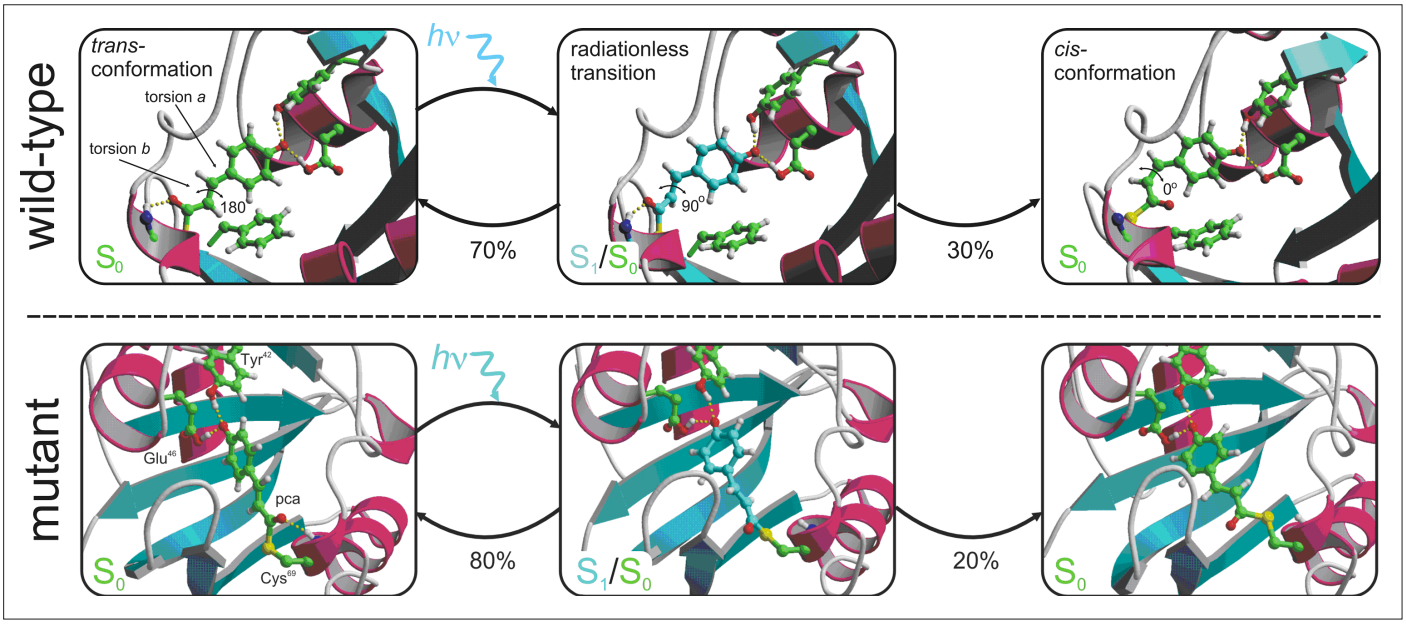
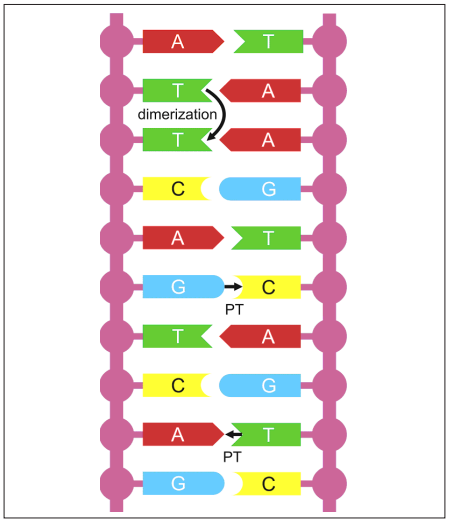


Fig. 1. Photochemistry of Photoactive Yellow Protein. Snapshots from excited-state trajectories of the wild-type (upper row), and mutant (lower row), showing the chromophore (pca) in the active site pocket. The first snapshot is at the excitation. The second shows the configuration at the radiationless transition from S_1 to S_0. The third snapshot shows the photoproduct. In the wild-type, isomerization takes place around the double bond (torsion *b*), whereas in the mutant, a single bond (torsion *a*) rotates. In both systems, the carbonyl oxygen of the thioester linkage flips, causing the break of the hydrogen bond to the backbone of Cys69.

Abb. 1: Photochemie des Photoactive Yellow Protein (PYP). Schnappschüsse aus einer Molekulardynamik-Trajektorie im lichtangeregten Zustand für den Wildtyp (oben) und für eine Mutante des Proteins (unten). Das Chromophor (pca) im aktiven Zentrum des Proteins ist gezeigt. Der erste Schnappschuss (links) ist bei der Lichtanregung. Der zweite zeigt das Protein beim Übergang vom lichtangeregten Zustand (S_1) zurück zum Grundzustand (S_0). Der dritte Schnappschuss zeigt das gebildete Photo-Produkt. Der Wildtyp rotiert um eine Doppelbindung (torsion *b*), während die Mutante um eine Einfachbindung (torsion *a*) rotiert. In beiden Proteinen rotiert das Carbonyl-Sauerstoffatom der Thioestergruppe, was zum Bruch einer Wasserstoffbrückenbindung zum Rückgrat der Aminosäure Cys69 führt.

To summarize, our results are consistent with experimental observations and provide detailed structural and dynamic information at a resolution well beyond that achievable by other means. From the simulations, we have identified the key amino acids and the mechanism by which they control the primary events in the photocycle of PYP. These are (i) photoisomerization around a double or a single bond, and (ii) the break of a hydrogen bond between the chromophore and the protein backbone. These events trigger a proton transfer from the protein to the chromophore, which ultimately leads to the signalling state of PYP.[2]



Photochemical Changes in DNA

Deoxyribonucleic acid (DNA) carries the genetic information of all cellular forms of life. DNA forms a double helix, in which the nucleoside bases of the single strands are stacked upon each other, forming strong hydrogen bonds with the bases in the complementary strand (Watson-Crick configuration). Due to the high absorbance of the bases in the harmful ultraviolet (UV) region of the spectrum, DNA is vulnerable to photochemical damage that can ultimately lead to cancer. To study the effects of UV radiation on DNA, we have performed molecular dynamics simulations of the ultrafast processes that follow photon absorption. As schematically shown above, we have considered two distinct types of reactions involving either bases in opposite strands or bases within the same strand.

The fastest photochemical reaction involving basepairs in opposite strands was found to be proton transfer.[6] Figure 2 shows the sequence of events that follows

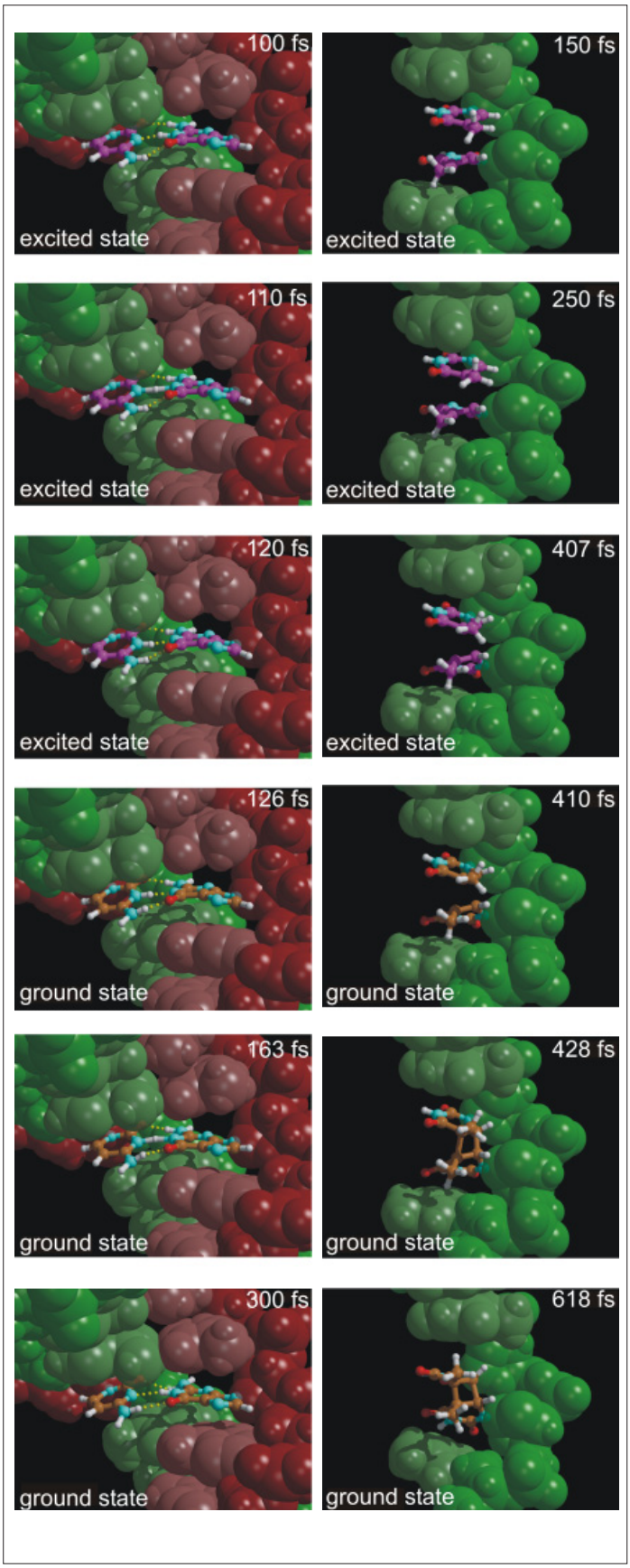


Fig. 2. Photochemistry of DNA. Excited state dynamics of a Watson-Crick cytosine-guanine (left snapshots) and a stacked thymine-thymine (right snapshots) basepair after light absorption. The left frames show the light-induced transfer of the central proton from a guanine to a cytosine. After this transfer, a radiationless transition to the ground state takes place (126 fs). The proton then rapidly returns to the guanine, restoring the Watson-Crick basepair (300 fs). The right frames show a photodimerization of two adjacent thymines. Decay to the ground state (410 fs) leads to the formation of a stable dimer.

Abb. 2: Photochemie von DNA. Dynamik im lichtangeregten Zustand eines Watson-Crick-Cytosin-Guanin-Basenpaares (Schnappschüsse links) und eines übereinander angeordneten Thymin-Thymin-Basenpaares (Schnappschüsse rechts) nach der Lichtabsorption. Die Schnappschüsse links zeigen den lichtinduzierten Transfer des zentralen Protons vom Guanin zum Cytosin. Nach diesem Transfer findet ein strahlungsloser Übergang vom angeregten Zustand zum Grundzustand statt (126 fs). Das Proton kehrt dann sehr schnell zum Guanin zurück, wobei das stabile Watson-Crick-Basenpaar wieder gebildet wird (300 fs). Die Schnappschüsse rechts zeigen die lichtinduzierte Dimerisierung zweier benachbarter Thymin-Moleküle. Beim Übergang vom angeregten Zustand in den Grundzustand (410 fs) wird ein stabiles Addukt gebildet.

excitation of a cytosine-guanine basepair in DNA. According to the simulations, photon absorption induces transfer of a proton from the guanine to the cytosine. This proton transfer enhances ultrafast decay of the excited state. After the radiationless transition to the ground state, the original Watson-Crick configuration is quickly restored. The entire photochemical reaction is completed within about 200 fs. The absorbed energy is converted into heat and therefore does not lead to structural damage. The same behaviour was observed for the adenine-thymine basepair.

In contrast to the benign inter-strand proton transfer, a reaction between adjacent bases in a single strand could lead to a more malign photoproduct. For example, intra-strand thymine dimerization is recognized as a common process leading to DNA damage induced by UV light. This mutagenic photoproduct can disrupt the function of DNA and thereby trigger complex biological responses, including apoptosis, immune suppression, and cancer. We have studied the photo-induced dimerization process in a single strand of DNA containing only thymines. As shown in Figure 2, the dimerization reaction occurs via a concerted mechanism on a sub-picosecond timescale.[7] This reaction only takes place if the thymine bases are distorted and close together. Such configurations, however, are very rarely observed in our MD simulations, accounting for the experimentally measured low quantum yield of dimer formation. The dimer forms a stiff kink in the double helix that can cause problems when the cell needs to replicate its DNA.

Thus also for the elucidation of the photochemistry in DNA, which is essential to understand why during evolution, DNA was selected to carry the genetic code, computer simulations can provide valuable insights at an atomic level. These insights will not only aid the interpretation of recent experiments, but also motivate new investigations. The applications that are presented here show what is feasible today with QM/MM molecular dynamics. The limits of today's computer technology restrict the system size that can be modelled. However, the expected increase of computer power will enable us to study larger systems and longer timescales in the near future, thereby broadening the applicability of this methodology.

Lars Schäfer studied Chemistry at the Technical University of Braunschweig. In 2003, he joined the group of Helmut Grubmüller at the MPIbpc and finished his PhD studies in June 2007. Currently, he is a Postdoc in the group.

Gerrit Groenhof studied Chemistry in Groningen, the Netherlands and obtained his PhD from the University of Groningen in 2005. He joined the group of Helmut Grubmüller at the MPIbpc as a Postdoc in 2005.

Helmut Grubmüller received his PhD in Theoretical Physics at the Technical University of Munich in 1994. During his time as Postdoc at the Theoretical Biophysics Group he made several research visits to the CENG in Grenoble, the University of Illinois and the ETH Zürich. In 1998, he became head of the Theoretical Molecular Biophysics Group at the MPIbpc, where he was appointed Director of the Department of Theoretical and Computational Biophysics in 2003. He is also Honorary Professor for Physics at the University of Göttingen.

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Zusammenfassung

Das Sonnenlicht als Energiequelle treibt viele biologische Prozesse an und ermöglicht so die Entwicklung des Lebens auf der Erde. Allerdings können hochenergetische ultraviolette (UV) Photonen auch strukturelle Schäden in Biomolekülen verursachen und so z.B. Krebs hervorrufen. Was aber passiert genau, d.h. auf der Ebene der einzelnen Atome, wenn Sonnenlicht auf Biomoleküle trifft?

Die Interaktion von Licht mit Materie ist außerordentlich komplex, und viele lichtgetriebene Prozesse laufen auf sehr schnellen Zeitskalen unterhalb einer Pikosekunde (10^{-12} s) ab. Die Dynamik derart schneller Reaktionen auf atomarer Ebene zu studieren ist derzeit selbst mit modernsten experimentellen Methoden nur sehr schwer möglich. Hier können atomistische Computersimulationen als mikroskopisches Gegenstück zu Experimenten detaillierte Einblicke liefern. Allerdings stellt auch die theoretische Modellierung lichtgetriebener Prozesse neue Herausforderungen, da sie methodisch anspruchsvoll und rechentechnisch aufwändig ist.

In diesem Beitrag berichten wir über unsere neuesten Arbeiten zur theoretischen Modellierung lichtgetriebener Prozesse in Biomolekülen. Wir untersuchen die atomaren Mechanismen zweier Prozesse, (i) des Mechanismus eines Photorezeptor-Proteins (Photoactive Yellow Protein, PYP), welches die Fluchtreaktion eines Bakteriums (*Halorhodospira halophila*) vor potentiell schädlichem Licht steuert, und (ii) licht-induzierter Prozesse in DNA. Unsere Simulationen helfen zu verstehen, warum DNA stabil gegenüber durch UV-Licht hervorgerufenen strukturellen Schäden ist, die zu Krebs führen können. Diese Stabilität ist wohl einer der Gründe dafür, dass sich DNA im Laufe der Evolution als Trägermolekül unseres Erbguts durchgesetzt hat.