Interplay between Structure, Electrical Fields and Biological Function

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Photobiological processes are part and parcel of biological life. The most famous and fundamental among them are vision and photosynthesis (fig. 1), but also less familiar processes like DNA repair and the circadian clock belong to this class. All photobiological processes have one thing in common : A constructive interaction between light and matter triggers biological function, conducted by the involved proteins. This initial light-matter interaction, however, takes place in small molecules, called chromophores. They have the ability to absorb light, and subsequently change their conformational structure. This initiates a cascade of structural changes which eventually involves the entire protein.

Fig. 1. Photosysthesis is one important photobiological process that renders life possible.

Bacteriorhodopsin

Our model system, the protein *bacteriorhodopsin*, is of special interest due to its very close analogy to *rhodopsin*, the primary light acceptor in human vision. Bacteriorhodopsin acts as proton pump incorporated into the membrane of the bacterium *halobacterium salinarum* (fig. 2). This

generated proton gradient serves the bacteria to establish metabolism. The function of both proteins is based on the chromophore retinal. Furthermore, bacteriorhodopsin represents a model for a large class of proteins, where the chromophore isomerizes. In the isomerization, part of the molecule is effectively rotating around a chemical bond leading to a new conformational structure of the molecule (fig. 3). The related time scales for this chemical process are in the range of a few hundred femtoseconds (1 fs = 10^{-15} s).

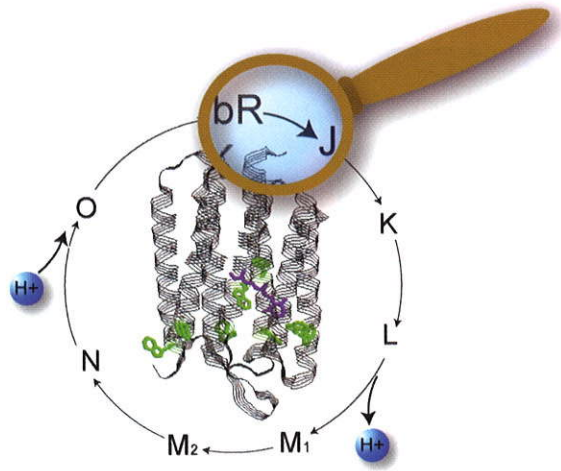

Fig. 2. During the photocycle, bacteriorhodopsin goes through different states (denoted by the letters). Eventually, the protein is back in its initial state (bR), while a proton is pumped across the membrane. The entire cascade of structural changes is initiated by the isomerization of retinal (state J).

Light initiated isomerization of the chromophore retinal is the first step of the photo-cycle and triggers the subsequent structural changes of the protein and the retinal itself – finally leading back to the initial state of the protein without the need of any secondary protein. This auto-regeneration and its high stability towards environmental conditions are advantageous properties of bacteriorhodopsin. In each run through the photocycle, one proton is pumped across the membrane. Turning over and over through this cycle, a difference in proton concentration is established.

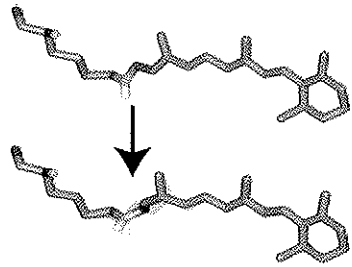

Fig. 3. Isomerization of the chromophore retinal is the first structural change triggering a series of modifications of the protein bacteriorhodopsin that lead to its biological function as a proton pump.

The motor : Light induced charge translocation

How exactly can light drive structural changes eventually leading to biological function ? Theoreticians developed a model to describe these first instants right after the absorption of light by the chromophore. In this model, the absorbed light is converted into electrical force by the rearrangement of charges on the chromophore. In consequence, this rearrangement drives the structural change of the chromophore itself. In addition, the constituents of the protein also respond to the charge redistribution in the so-called dielectric response. Eventually, these initially small changes are amplified to significant changes in the protein structure, which then drive the biological process. In a nut shell, the light-induced charge redistribution ignites the cascade of the related biological process. Up to now, this charge redistribution was inaccessible to experimental approaches.

Intrinsic voltmeter with ultrafast and nanometer resolution

To measure the fast initial charge redistribution on the retinal, a highly specialized voltmeter is necessary. First, this voltmeter must be able to measure electric fields on the dimensions of subnanometer, as the retinal measures only about 1 nm. Additionally, the voltmeter must provide an extremely high time resolution. The isomerization occurs on a time scale of 200 femtoseconds, i.e. $200 \cdot 10^{-15}$ seconds. To observe the charge translocation, the time resolution has to be far better than that value.

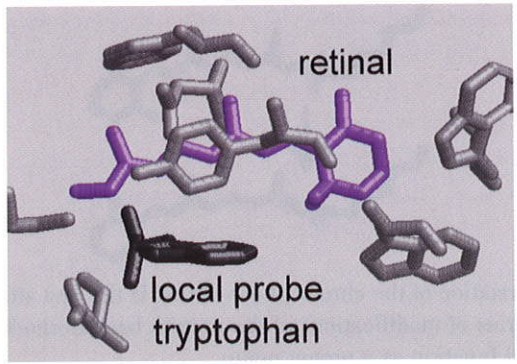

Fig. 4. The amino acid tryptophan is an ideal probe for transient short-lived electrical fields inside proteins : it is small and naturally present in most proteins. In bacterio-rhodopsin, two tryptophans are located in the immediate vicinity of retinal, and can act as reporters of electric field changes.

At this point, we were able to overcome this experimental challenge, by identifying an excellent natural voltmeter on a nanometer scale - the aminoacid typtophan. It is a small chromophore, and it has a well accessible absorption and emission window in the near-ultraviolet spectral region. Most important, it is naturally present and abundant in most proteins. In bacteriorhodopsin, one tryptophan is located in the next vicinity of the retinal (fig. 4). Tryptophan is highly sensitive to electric fields (fig. 5). Variations in the surrounding charge distribution are converted into variations of shape, intensity and maximum position

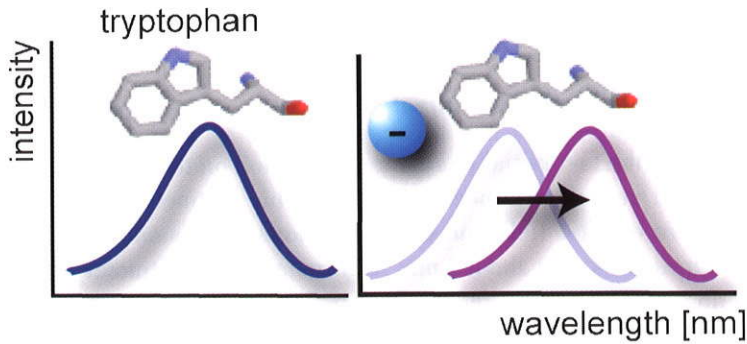

Fig. 5. Variations of the electric charge in proximity of tryptophan induce a wavelength shift of the absorption spectrum.

of the absorption and emission spectra. It is exactly this property that allowed us to monitor of the charge redistribution on the neighbouring retinal by optical spectroscopy. Thus, to read out the voltmeter, we use optical laser spectroscopy with the required time resolution of femtoseconds, fast enough to monitor these ultrafast processes.

The time-resolved measurement is based on the use of two laser pulses, the so-called pump pulse excites the retinal, and the second (called probe) pulse reads out the absorption changes of the tryptophan. By varying the optical path of the probe pulse, the time delay between these two laser pulses can be changed with femtosecond time resolution.

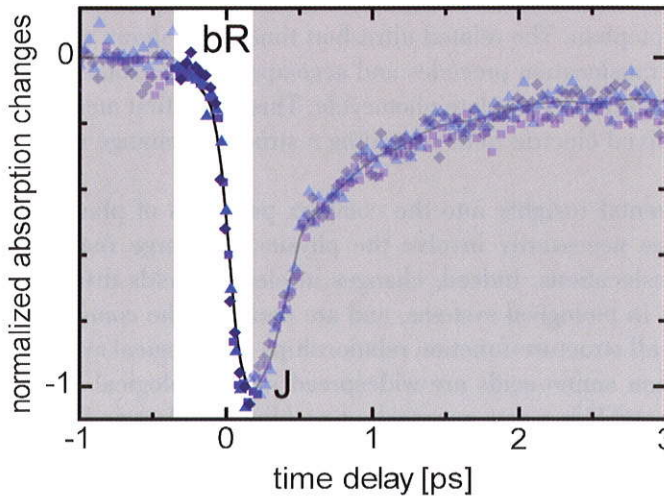

Fig. 6. First observation of the ultrafast initial translocation of charge inside a protein monitored by the absorption changes of tryptophan : The decrease in absorption reflects an increase in electric field strength over 200 fs.

First experimental evidence for an initial charge translocation within a protein

Indeed, the measured absorption difference signal on the local probe tryptophan unravels the initial charge translocation on the retinal (fig. 6). The absorption decrease is directly related to an increasing charge separation on the retinal, which we confirmed by a theoretical

model. This means that in the first 200 fs the molecule is more and more electrically polarized, i.e. positive and negative charges become more and more separated on the molecule. The following recovery of the absorption difference signal is connected to the isomerized retinal, which exhibits a different charge distribution than the ground state. The measurement was cross-checked on a mutant of bacteriorhodopsin, leading to similar results and reinforcing our theoretical model.

Conclusion and impact : beyond fundamental research

In conclusion, we observed for the first time the initial translocation of charge in a biological chromophore by addressing the absorption changes of an intrinsic nanoscale voltmeter of the protein, the amino-acid tryptophan. The related ultrashort time scale shows well, that this charge translocation precedes and accompanies the isomerization process, triggering a complete photocycle. This is the first measurement of a short lived electric field preceding a structural change within a protein.

Fundamental insights into the complex pathways of photo-biological processes necessarily involve the physics of charge rearrangements and translocations. Indeed, changes in electric fields drive structural changes in biological systems, and are therefore the common denominator to all structure-function relationships in biological systems. Since tryptophan amino-acids are widespread in all biological systems, our results establish a new approach at probing the electric field changes within them. The ability to observe and measure these electric fields yields another dimension to our understanding of how nature works.