Dynamics of biomolecules in the gas phase: Isomerization of retinal as bridge from *in vacuo* to *in vitro* photophysics.

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The structure and dynamics of bio-active molecules are "hot" topics in modern photon science since understanding nature's chemistry in the small size limit under well-defined conditions will provide key information on metabolic processes in large biomolecular systems and will lead in the final step to a real time observation of changing structure during an ongoing biochemical reaction. Many processes occurring in nature upon electronic excitation proceed on the ultrafast fs timescale while subsequent changes of the bio-molecule's coordination, which determines its biological function, are based on picosecond and longer timescales. Femtosecond laser pulses in a pump-probe experimental setup address the initial ultrafast dynamics.

An important biological mechanism is the photosynthesis cycle of Bacteriorhodopsin (bR), that converts optical light energy into chemical energy by generating a proton gradient and serves as the primary event to vision in higher organisms and photoreceptor response. The protein's biological activity given by its structural dynamics is determined to a large extent by the light absorbing chromophore retinal in its protonated Schiff-Base form (340 amu). After photo absorption from the electronic ground state (S_0) the retinal cation isomerizes from an all-trans state into a cis configuration around the C13-C14 bond as an initial step in the photocycle. Although structurally well characterized the dynamics on the atomic scale demand clarification.
The trans-cis isomerization time of the chromophore is fast in proteins (some hundred femtoseconds [1]) but about a magnitude slower in solutions as methanol [2]. In the first 200 fs a skeletal change of the linear C-chain occurs, that triggers the nuclear motion followed by isomerization and slower vibrational relaxation of the cis-photoproduct. It is not known yet, if the fast response is an intrinsic property of retinal or a result of particular electrostatic interaction with a few number of water molecules in the vicinity of the chromophore given that the high dielectric constant in water stabilizes charge separation and can greatly enhance or suppress chemical reactions.

To eliminate perturbations introduced by the local environment we performed measurements of the isomerization dynamics of the bare protonated retinal in vacuum for the first time. The Schiff-Base cations were brought into the gas phase by an electrospray-ionization source using a 100 μmol/l solution of retinal in methanol. Via photon absorption from the electronic ground state at a central wavelength of 600 nm ($S_0 \longrightarrow S_1$) and a pulse energy of 1 - 10 μJ in the pump step disintegration due to ionization is absent and isomerization around the C13-C14 bond into the cis-configuration is triggered as shown in Fig.1. The soft X-ray pulse of FLASH Coulomb explodes the photoproduct. The kinetic energy distribution of retinal fragment ions generated by the FEL probe pulse shall monitor the transient geometric structure change during the isomerization reaction triggered by the optical laser. This is because the average spatial distances of charge states differ in trans- and cis-configurations, respectively. FLASH pulses of 7 nm wavelength (ca. 177 eV) have been used since special multilayer optics with small bandwidth (< 0.5 eV) were used to get rid of wavelength fluctuations. In the first part of our measurement campaign in February 2011 we evaluated the drift

times of all fragment ions that were produced during Coulomb explosion. As the retinal cations are a very dilute target not only the fragments originating from this species, but also all fragment ions of the former neutral solvent molecules, mainly methanol contribute to the signal.

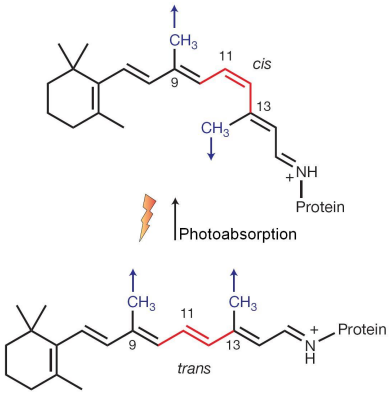

Figure 1: Trans-cis isomerization of retinal. After photoexcitation an isomerization from the trans- to the cis-configuration around the C13-C14 bond in the C-chain occurs. [3]

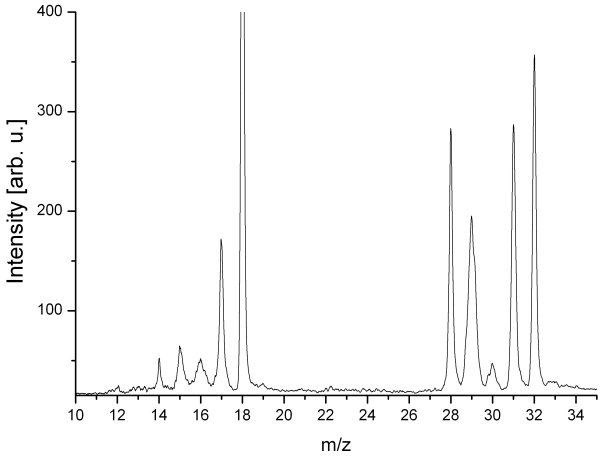

Figure 2: Mass spectrum of fragment ions from retinal and solvent after FEL-probe pulse.

For the second part of our beam time in summer 2011 the kinetic energy detection was improved by using a mass-selecting time-of-flight (TOF) spectrometer. Using a temporal gating it was furthermore possible to not only observe expected kinetic energy distributions from all fragment ions at once, but to distinguish between the different fragments' mass/charge ratio (m/z) as can be seen in Fig.2. It shows a typical mass spectrum after the FEL-triggered Coulomb explosion. Nevertheless the effect of the background (solvent) fragment ions is still dominant as mentioned before. To discriminate the photoproduct's influence additional characterization measurements of the molecular beam are currently performed. These measurements are important for future experimental campaigns where the influence of a controlled number of added water molecules to the bare retinal shall bridge the gaps from *in vacuo* via *in vitro* to *in vivo*.

References

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