DETERMINATION OF SEQUENCE-SPECIFIC DNA STRAND BREAKS INDUCED BY VUV RADIATION USING THE DNA ORIGAMI TECHNIQUE

S. Vogel[1], J. Rackwitz[1], R. Schürmann[1, 2] J. Prinz[1], A. R. Milosavljević[3], A. Giuliani[4] and I. Bald[1, 2]

[1]*Institute of Chemistry, University of Potsdam, Karl-Liebknecht-Str. 24-25, 14469 Potsdam, Germany*
[2]*BAM Federal Institute for Materials Research and Testing, Richard-Willstätter Str. 11, 12489 Berlin, Germany*
[3]*Institute of Physics Belgrade, University of Belgrade, Pregrevica 118, 11080, Serbia*
[4]*Synchrotron SOLEIL, Gif-sur-Yvette, France*

Abstract. We have studied the sequence-specific DNA damage induced by VUV photons in an energy range from 6.5 eV to 9.5 eV on the single-molecule level. By using a novel DNA origami technique, we were able to visualize the photon-induced dissociation of single chemical bonds within complex but well-defined self-assembled DNA nanostructures, deposited on a UV transparent substrate. The method employs atomic force microscopy (AFM) to image and quantify photon-induced bond dissociations within specifically designed oligonucleotide targets that are attached to DNA origami templates. Therefore, we were able to determine quantitatively the sequence-specific DNA strand break yields in dependence of the VUV photon energy.

1. INTRODUCTION

There is a long standing effort to fully understand photon interaction with DNA and RNA molecules, which are carriers of genetic information. DNA and RNA strongly absorb in ultraviolet (UV) and far-UV spectral region [1], which should lead easily to photon-induced destruction or severe carcinogenic damage. However, it appears that there is an amazing photostability of DNA, suggested to be due to an ultra-fast de-excitation of the bases from their excited electronic state to a hot ground state, thus allowing an efficient cooling of the molecule through vibrational interactions with the environment. Nevertheless, an UV-induced ultrafast damage of oligonucleotides can occur by formation of cyclobutadiene pyrimidine dimers [2], and at photon energies higher than 8 eV DNA strand breaks in plasmid DNA have been observed [3]. All these results led

to better understanding of very complex processes triggered by energetic photon interaction with DNA, which depend on physicochemical properties of single bases, their inter-connections, solvent effects etc. [1]. However, it has not been possible so far to visualize and quantify photon-induced damage to oligonucleotides of specific nucleotide sequence on a single molecular level. Stacking interactions between the DNA nucleobases lead to a strong modification of the nucleobase's electronic properties and hence their photoinduced fragmentation dynamics [4]. Measuring the photon-induced DNA strand breaks on a single molecular level as a function of the photon energy and the flux is particularly interesting for both the studies on DNA photostability and the important field of radiation damage research [3,5].

We have recently developed a novel method that allowed for the first time to visualize the electron-induced dissociation of single chemical bonds within complex, but well-defined self-assembled DNA nanostructures [6,7]. We use AFM to image and quantify low-energy-electron-induced bond dissociations within specifically designed oligonucleotide targets that are attached to DNA origami templates thereby forming a DNA nanoarray. In electron-irradiation experiments we have used 18 eV electrons and found that at a fluence of 1 – $5x10^{12}$ cm^{-2} the number of DNA strand breaks increases linearly with the electron fluence. By using electron beams with a current of 1 – 10 nA irradiation times below 100 s are required. This novel technique enables the fast and parallel determination of DNA strand break yields with unprecedented control over the DNA's primary and secondary structure.

In the present work, we combine the DNA origami technique with the wide-energy high-brilliance VUV synchrotron source at SOLEIL, in order to perform a detailed investigation of DNA photo-radiation damage in its most natural environment and on a single molecule level.

2. EXPERIMENTAL METHOD

2.1 DNA origami samples

DNA origami nanostructures were prepared from the M13mp18 scaffold strand and a set of 208 short oligonucleotides according to a well-established procedure [6]. In brief, the DNA strands are mixed in TAE buffer with 10 mM $MgCl_2$ and annealed from 80°C to room temperature within 2 hours, and the non-assembled excess strands are removed by spin-filtering. The assembled structures are deposited on SiO_2/Si and CaF_2 substrates, respectively, by deposition in 10x TAE buffer with 100 mM $MgCl_2$ solution for 45 min. The excess solution is removed by washing with 4 mL of ethanol/water (1/1) mixture and subsequently the sample is dried with a blow of nitrogen. Then the samples are transferred into the chamber filled with Ar and irradiated with VUV photons with defined energy and fluence. After irradiation the samples are washed again with ethanol/water to remove fragmentation products and then incubated in a 50 nM solution of

streptavidin for 5 min, washed again and dried. Then the samples are analyzed with AFM.

2.2 Experimental set up

The irradiation chamber of the beamline is connected to the APEX branch of the DISCO beamline at the synchrotron SOLEIL synchrotron radiation facility. The samples were deposited on a holder attached to a Z manipulator that allowed for fine adjustment of the sample on the beam. The differential pumping system of the beamline was used to filter out the second order light. The samples were irradiated for different time to establish a dose-response curve as a function of the photon energy. The photon flux was measured using a calibrated photodiode.

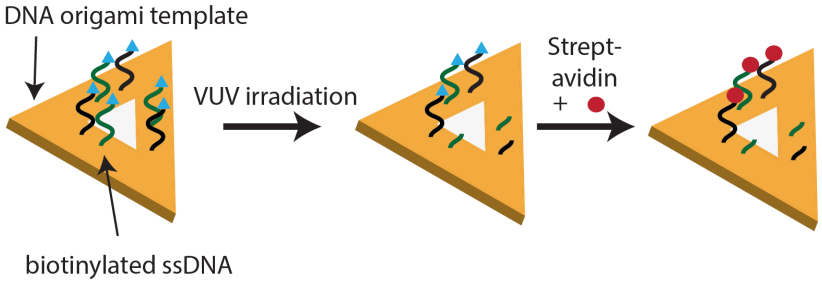

Figure 1. Scheme of the DNA origami triangles, which serve as a support of the oligonucleotide target structures. After the irradiation with VUV photons the remaining intact target structures are visualized for AFM using streptavidin.

3. RESULTS

We have irradiated DNA origami samples carrying the target sequences 5'-TT$(XTX)_3$TT (X = C,A) with VUV photons at 6.5 eV, 7.3 eV, 8.44 eV, 8.94 eV, 9.14 eV and 9.5 eV. By using AFM for the visualization of DNA single strand breaks in the target sequences we could observe that the relative number of DNA strand breaks increases with the photon flux. Thus, we could establish dose-response curves for all photon energies. The energies were chosen to match either resonances observed in previous experiments (6.5 eV and 7.3 eV)[9],[10], or the ionization potentials (IP) of isolated nucleobases (IP(A) = 8.44 eV, IP(C) = 8.94 eV, IP(T) = 9.14 eV), or to be right above the ionization threshold of all DNA bases (9.5 eV). By comparing the DNA strand break yield of the two target sequences at the different irradiation energies, we can deduce important information about the underlying damage mechanism.
Furthermore, we performed measurements on both SiO_2/Si substrates and VUV transparent CaF_2 to directly compare the effect of secondary electrons released from the Si substrate. All measurements have been performed at atmospheric pressure under Ar to avoid DNA damage induced by evacuation or venting of the chamber.

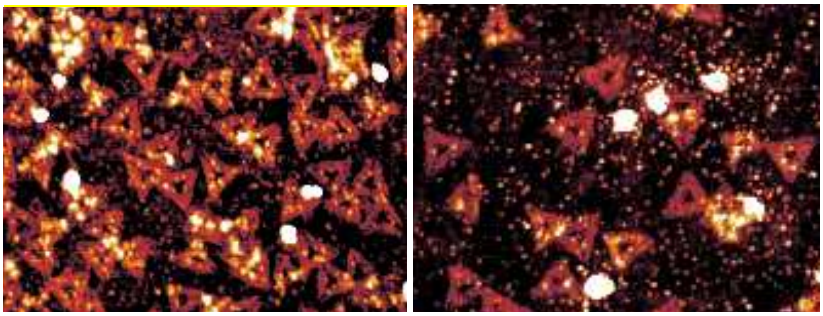

Figure 2. AFM images of DNA origami nanostructures deposited on CaF_2 irradiated at 8.44 eV for 1 min (left), and 20 min (right).

Acknowledgements

This research was supported by a Marie Curie FP7 Integration Grant within the 7th European Union Framework Programme and by the Deutsche Forschungsgemeinschaft (DFG). We are grateful to the synchrotron SOLEIL for providing beamtime and support under project no. 20131271 and to a grant by the German Academic Exchange Service (DAAD, project No. 57055766) supported by the German Federal Ministry of Education and Research. A.R.M. acknowledges support by the MESTD of RS (171020) and the COST Action MP1002 (Nano-IBCT).

REFERENCES

[1] Middleton et al, Annu. Rev. Phys. Chem. 60, 217 (2009).
[2] W. J. Schreier, T. E. Schrader, F. O. Koller, P. Gilch, C. E. Crespo-Hernandez, V. N. Swaminathan, T. Carell, W. Zinth, B. Kohler, Science 315, 625 (2007).
[3] K. Hieda, Int. J. Radiat. Biol. 66, 561, (1994).
[4] U. Kadhane, A.I.S. Holm, S.V. Hoffmann, S.B. Nielsen, J. Photochem. Photobiol. A 197, 110 (2008).
[5] I. Baccarelli, I. Bald, F. A. Gianturco, E. Illenberger, J. Kopyra, Phys. Rep. 508, 1 (2011).
[6] A. Keller, I. Bald, A. Rotaru, E. Cauet, K. V. Gothelf, F. Besenbacher, ACS Nano 6, 4392 (2012).
[7] A. Keller, J. Kopyra, K. V. Gothelf, I. Bald, New J. Phys. 15, 083045 (2013).
[8] Smialek et al, Radiat. Res. 172, 529 (2009).
[9] Smialek et al, J. Phys. Conf. Ser. 101, 012020 (2008).
[10] A. R. Milosavljević, V. Z. Cerovski, F. Canon, M. Ranković, N. Škoro, L. Nahon, A. Giuliani: J. Phys. Chem. Lett. 5, 1994 (2014).