Low-Energy (5 – 20 eV) Electron Induced Single and Double Strand Breaks in Well-Defined DNA Sequences

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Preparation and irradiation of DNA origami structures

DNA origami triangles are prepared in a one pot synthesis in a self-assembly process of the M13mp18 phage scaffold strand and 208 well designed staple strands. The DNA origami solution is washed afterwards and stored in 1 x TAE buffer with 12.5 mM $MgCl_2$ for stabilisation. Subsequently, dried DNA origami nanostructure samples on plasma cleaned silicon wafers are mounted on the sample stage of a customized high vacuum irradiation chamber. The low-energy electron (LEE) irradiation is operated at a pressure of 10^{-8} mbar produced by a turbo molecular pump from Agilent (TwissTorr 304 FS). The LEE-beam with a specific electron energy (5 to 20 eV) with a resolution of 1.2 ± 0.1 eV is centred on the samples. Fluences of $4 * 10^{13}$ cm^{-2} are used during the irradiation procedure to avoid exponential saturation of DNA strand breaks at higher fluences with currents in the low nanoampere range of 2-5 nA with irradiation times of 10, 20, 30,

40 ,50 and 60 seconds. Working in a low fluence regime results in a linear exposure-response curve and two-electron processes become very unlikely. At least two to three series of six samples each are irradiated and compared with the two non-irradiated control samples inside and outside the irradiation chamber. A total of 16 to 24 DNA origami nanostructure samples are analysed in detail via atomic force microscopy (AFM). A variation of N_{SB} at zero fluence from batch-to-batch is found and is ascribed to a variation of the SAv quality and the quality of target strands (e.g. the yield of successfully coupled Bt). Nevertheless, the determination of the strand break cross sections only requires the determination of the slope, i.e. relative changes with respect to the control sample. Therefore, within one series of irradiations all the sample conditions were the same to ensure that the sensitivity of the method is not compromised.

AFM data analysis and determination of absolute DNA strand break cross section

The samples are analysed within the center of the irradiation area using atomic force microscopy in order to make a correlation between the measured current and the calculated fluence. At least four AFM images (4 x 4 µm² with 1024 pixel/line) are taken from each sample (32 images/series) including a total of about 8000 DNA origami nanostructures per series. In total, a set of 16000 to 24000 DNA origami nanostructures have to be analysed manually. These large set of data reflects good statistics and a meaningful result with a small error. Intact DNA strands are visable as bright spots in the AFM images and can be manually counted giving the number of strand breakage N_{SB} calculated from the number of intact strands N and the total amount of possible DNA strands N_0 ($N_{SB} = 1 - (N/N_0)$). The determination of absolute cross sections of LEE induced DNA strand breaks σ_{SB} is achieved from the linear fit of the number of strand breakage N_{SB} as a function of the fluence

F. Each DNA origami triangle can carry two different DNA target sequence and can be directly compared under the same experimental conditions

FRET experiments for control of double strand formation

For the experiments described in the text the successful formation of a double strand must be ensured. Therefore, dye labeled DS_{dye} (5'- d$(CAC)_4$-Cy3 and 3'- d$(GTG)_4$-FAM) are placed next to each other on the DNA origami triangle (figure 1a iv). The dye functionalization enables the observation of Förster resonance energy transfer (FRET) between a donor (fluorescein; FAM) and an acceptor dye (cyanine3; Cy3) attached to the ends of the complementary single strands. FRET is a mechanism describing an energy transfer via nonradiative dipole-dipole coupling between two light-sensitive chromophores, which is highly distance-dependent. An overlap of the emission spectrum of the donor with the absorption spectrum of the acceptor dye is required to guarantee an energy transfer. The formation of a DNA double strand of DS_{dye} allows an energy transfer resulting in a characteristic FRET signal in fluorescence emission spectra by exciting the donor dye (450 nm). Figure S1c shows the emission spectra of the ssDNA $(GTG)_4$-FAM excited at 450 nm with a maximum at 515 nm (yellow line) and $(CAC)_4$-Cy3 excited at 500 nm with a maximum at 565 nm (red line). Both, free in solution and attached to the DNA origami, no differences in the emission spectra of $(GTG)_4$-FAM and $(CAC)_4$-Cy3 are observed. In contrast to this, the double stranded DS_{dye} free in solution (figure 1c black dotted line) and attached to the DNA origami (Figure S1d black dotted line) show different emission spectra. By exciting the free DS_{dye} at 450 nm, the emission of Cy3 increases at 565 nm. This indicates a successful energy transfer over small distances (1 to 10 nm) between the donor and acceptor dye. If the DS_{dye} is bound to the DNA origami triangle, only a weak energy transfer is observed. This is ascribed to the fact that the two

ssDNA did not hybridize when attached to the DNA origami triangles. Moreover, the two single stranded DNA can hinder each other in the folding process because of their close proximity. As a result, only one of the two single stranded DNA could be bound to the DNA origami. Additionally, electron induced DNA strand break experiments of single stranded DNA $ssDNA_1$ 5'-Bt-d$(CAC)_4$-Bt and $ssDNA_2$ 5'-d$(GTG)_4$-Bt (Figure S1a ii and iii) and double stranded DNA using complementary single stranded DNA DS (5'-Bt-d$(CAC)_4$-Bt / 5'-d$(GTG)_4$-Bt) (figure 1a i) supports the FRET experiments. Almost identical σ_{SB} are obtained for irradiated DNA oligonucleotides $ssDNA_1$, $ssDNA_2$ and DS at an electron energy of 10 eV (Figure S1b).

Therefore, the formation of the DNA double strand is ensured by a DNA hairpin hpDNA (5'- d$(CAC)_4$T(Bt- dT)$T_2(GTG_4)$ (see Figure 1b in the main manuscript). During the annealing process, the DNA hairpin forms a double strand consisting of twelve DNA base pairs and a loop of four non-hybridized thymine bases. The Bt label is covalently bound to one of the thymine bases in the DNA hairpin loop. It was shown that the Bt label is subject to LEE induced DNA damage itself, which has to be considered in the final strand break yield.

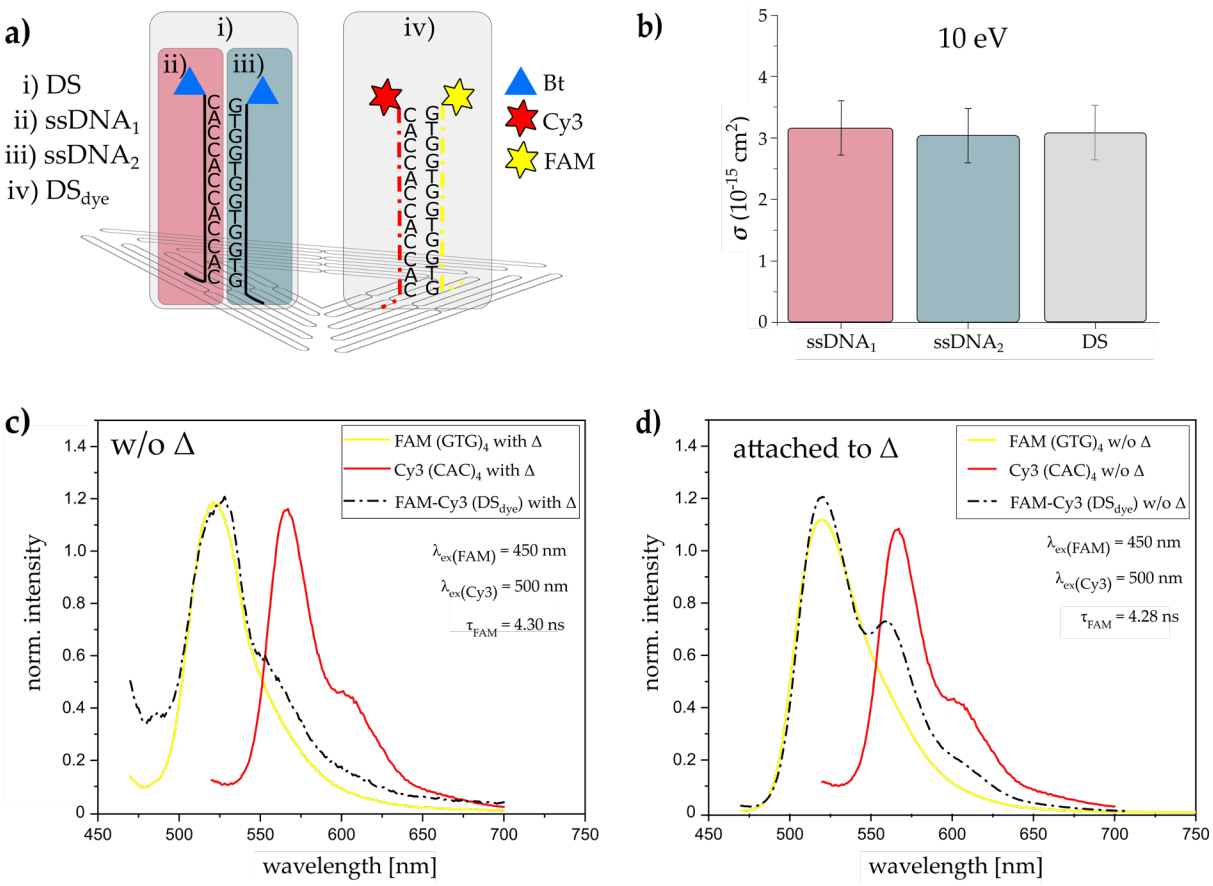

Figure S1 a) Illustration of the DNA origami triangle modification with i) the stem sequence DS (5'-Bt-d(CAC)$_4$ / 5'-d(GTG)$_4$-Bt), ii) the single strand ssDNA$_1$ 5'-Bt-d(CAC)$_4$, iii) the single strand ssDNA$_2$ 5'-Bt-d(GTG)$_4$ and iv) the dye modified double strand DS$_{dye}$ (5'-Cy3-d(CAC)$_4$ / 5'-d(GTG)$_4$-FAM). DS modification is used in the LEE induced DNA irradiation experiments. b) Absolute DNA strand break cross sections σ_{SB} for the DNA single strands (5'-Bt-d(CAC)$_4$-Bt and 5'-d(GTG)$_4$-Bt) and the DNA double strand DS (5'-Bt-d(CAC)$_4$-Bt / 5'-d(GTG)$_4$-Bt). Determined σ_{SB} [10^{-15} cm^2] for LEE irradiation at 10 eV: ssDNA$_1$ (3.10 ± 0.41), ssDNA$_2$ (3.00 ± 0.32) and DS (3.08 ± 0.36). c) Results of the two-dye FRET system with FAM and Cy3 as donor and acceptor not attached to the DNA origami triangle. The normalized fluorescence emission spectra of the single stranded DNA (GTG)$_4$-FAM (yellow; excited at 450 nm; maximum at 515 nm) (CAC)$_4$-

Cy3 (red; excited at 500 nm; maximum at 565 nm) and the hybridized double stranded DNA DS_{dye} (black dashed line; excited at 450 nm). The emission spectra of DS_{dye} show the emission of FAM and Cy3 due to energy transfer from donor to acceptor dye. d) Two-dye FRET system with FAM and Cy3 modified DNA target sequences $(GTG)_4$-FAM and $(CAC)_4$-Cy3 as donor and acceptor attached to the DNA origami triangle. The emission spectra of DS (dashed line; excited at 450 nm) show the emission of FAM at 515 nm and only a small increase of Cy3 emission at 565 nm. This indicates a less favorable hybridization of DS compared to DS_{dye} when not attached to the DNA origami triangle.

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