Report on the outcomes of a Short-Term Scientific Mission[1]

Action number: CA20129

Grantee name: Leo Sala

Details of the STSM

Title: High LET irradiation of DNA-based data carrying nanostructures

Start and end date: 02/05/2025 to 06/05/2025

Description of the work carried out during the STSM

Description of the activities carried out during the STSM. Any deviations from the initial working plan shall also be described in this section.

The irradiation experiments at the D1 beamline of GANIL proceeded as planned and were completed within the allotted beam time, with sufficient buffer time to accommodate for beam tuning and dosimetry, unexpected technical issues, and associated troubleshooting.

A $^{16}O^{8+}$ ion beam with 95 MeV u^{-1} energy and dose rates of 4–7 × 10^8 ions cm^{-2} s^{-1} were used to irradiate three patterns of data-carrying rectangular DNA origami nanostructures in aqueous solutions of various concentrations of radical scavengers, contained in 1.5 mL Eppendorf tubes mounted, and mounted on a dedicated sample holder. Samples with higher radical scavenger concentrations were irradiated with fluences ranging from 1–100 × 10^{10} ions cm^{-2}, corresponding to absorbed doses in water of 0.8–78 kGy. In contrast, those with lower radical scavenger concentrations were irradiated with fluences of 1–20 × 10^{10} ions cm^{-2} (0.8–16 kGy).

Due to the high irradiation doses, the samples could only be retrieved 24 hours post-irradiation, as the exposed area—including the sample holder—had become activated. Stringent radiation safety checks were required to confirm that residual radioactivity was below safety thresholds before sample recovery. After retrieval, a 1 µL drop from each tube was deposited on-site onto plasma-cleaned Si chips (~ 0.5 x 0.5 cm2) for subsequent AFM analysis, while the remaining sample volume was stored at 4 °C for transport. These samples will be further analyzed using UV-Vis spectrophotometry and gel electrophoresis. Although the host institution is equipped with the necessary characterization tools, due to the high number of samples irradiated (including replicates), the post-irradiation analyses will be mostly carried out in our home laboratory in Prague to complete the STSM at the host institution within the planned duration of stay.

[1] This report is submitted by the grantee to the Action MC for approval and for claiming payment of the awarded grant. The Grant Awarding Coordinator coordinates the evaluation of this report on behalf of the Action MC and instructs the GH for payment of the Grant.

Description of the STSM main achievements and planned follow-up activities

Description and assessment of whether the STSM achieved its planned goals and expected outcomes, including specific contribution to Action objective and deliverables, or publications resulting from the STSM. Agreed plans for future follow-up collaborations shall also be described in this section.

The primary objective of the STSM was to estimate the upper limits of radiation-induced damage to data-carrying DNA nanostructures. Preliminary AFM characterization of irradiated samples enabled the identification of dose ranges in which structural damage to these nanostructures occur. Figure 1 presents one such irradiated DNA nanostructure, featuring bit patterns in the upper left corner (Fig. 1A). Averaged AFM images of individual nanostructures exposed to increasing doses of a 95 MeV·u^{-1} ^{16}O ion beam (Fig. 1B). Reduction in the average intensity/height of the protein-functionalized bit-coded region becomes apparent between 2 and 8 kGy of absorbed dose, with a 15% signal loss observed at 8 kGy (Fig. 1C). Another pattern (Fig. 2A), irradiated up to 16 kGy, shows feature degradation in the upper region of the nanostructure. Pronounced alterations in the bit patterns are thus observed between 8 and 16 kGy. Nevertheless, considering that background radiation typically results in exposures in the mGy/year range, these dose levels correspond to timescales of millions of years, indicating the potential of these nanostructures for long-term data storage. By increasing the concentration of radical scavengers, this radiation tolerance can be extended up to 40–80 kGy. This could further extend the applications of the method to environments with high radiation exposure (e.g. space).

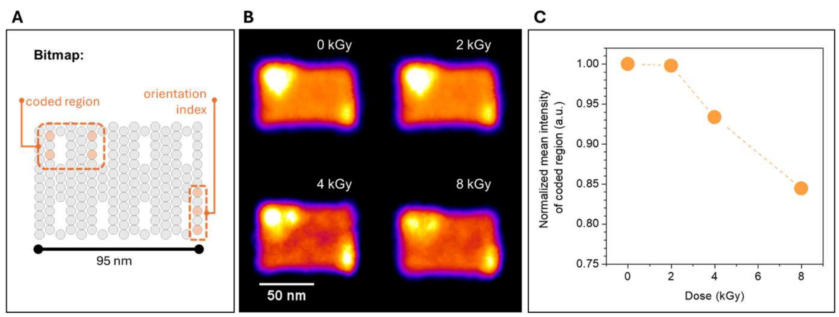

Figure 1. A bit pattern on a DNA nanostructure (A) with the average of stacked AFM images in (B) at different absorbed doses of ion beam irradiation. The normalized intensity of the bit-coded region is plotted against absorbed dose in (C).

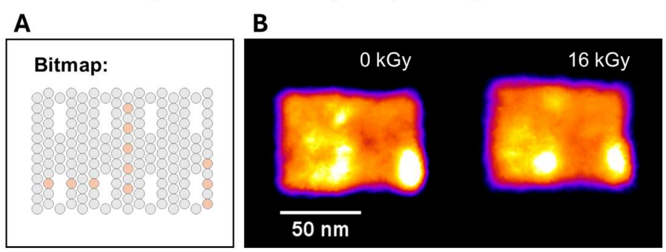

Figure 2. Another bit pattern (A) with average AFM images of the control and 16-kGy ion beam-irradiated sample in (B).

The AFM images shown in Fig. 1-2 were acquired at lower resolution; higher-resolution scans are currently in progress to better resolve the bit patterns and enable a more quantitative assessment of bit loss from radiation exposure. Full characterization of samples at intermediate doses is also underway. Preliminary observations suggest that the samples remain in good condition and that the irradiation experiments were precisely conducted. This is supported by the intrinsic Tris-based dosimetry present in the buffer, which shows consistent, dose-dependent responses (Fig. 3). The results obtained from this STSM will be compared with those from electron (low LET) irradiation experiments (also in progress) to develop a more comprehensive understanding of total ionizing dose effects on data-carrying nanostructures.

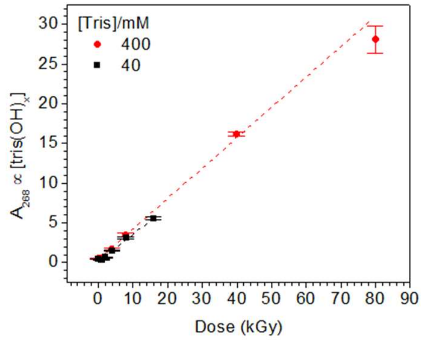

Figure 3. Absorbance at 268 nm of the data-carrying DNA nanostructure solutions with respect to absorbed dose.

Overall, based on the early results, the main goals and expected outcomes of the STSM have been reached and will be further improved once the post-irradiation analyses are completed. This work supports the efforts of WG 1, which focuses on chemical changes caused by irradiation in complex molecular systems/biomolecular structures, especially to Task 1.2, which looks at how ion irradiation affects complex biomolecules like DNA origami. The findings might also be useful for Task 1.5 (MultIChem database), which compiles information on chemical changes caused by irradiation—particularly in this case, those involving ion beams and DNA/DNA-based nanostructures.