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

Action number: CA20129 MutIIChem

Grantee name: Dorothea C. Hallier

Details of the STSM

Title:Irradiation of ssDNA-binding proteins with gamma rays

Start and end date: 23/06/2025 to 04/07/2025

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.

Before the official start of the STSM, the delicate Gene-V Protein (G5P) protein was expressed and purified in the applicants home lab at Fraunhofer IZI-BB in Potsdam, Germany. The concentration of G5P in solution was 1 mg/mL. The protein was then shipped on dry ice to Université Paris-Saclay, Orsay, France.

Irradiations of G5P with and without the OH-radical scavenger TRIS were performed at 15, 50 and 100 Gy with gamma rays in PBS and phosphate buffer under nitrogen, nitrogen-oxygen (4:1) and air conditions.
Mass spectrometry experiments with TOF were performed after a post-irradiation treatment with trypsin.
The data was analyzed regarding alterations at aromatic (Tyrosine and Phenylalanine) and positively charged (Lysine and Arginine) as well as sulphur containing (Cysteine and Methionine) amino acids depending on the applied conditions (radiation dose, atmospheric conditions, presence of scavenger).

Additional irradiations and respective post-irradiation analysis were performed on smaller peptides containing the relevant amino acids mentioned above under the same conditions to be able to study the impact of the used buffer and the influence of the structure in detail.

[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 STSM titled „Irradiation of ssDNA-binding proteins with accelerated gamma rays“ has successfully achieved all of its planned goals and expected outcomes. The STSM extended the already existing research in the COST Action MultIChem with never-before collected experimental data on the radiation damage of ssDNA-binding proteins with gamma rays.
All of the initially planned experiments were performed.

Relevant alterations to G5P and the studied amino acid residues were detected upon irradiation with gamma rays. Specific trends were detected regarding the applied conditions (dose, atmosphere) considering oxidations of the relevant amino acids and specifically the dimerization of Tyrosine residues.

The experimental results are yet to be analyzed and understood in detail. Additional MS measurements are planned and thorough analysis of the additional peptide data are to be performed.

The activity control of the irradiated protein will be performed in the home lab of the applicant in Potsdam Germany.

The collaboration between the applicants and the host institutions will be continued.
There are several plans to analyze the experimental results of this STSM. For example, additional irradiations with other scavengers, different buffers, different doses or in addition of DNA could be performed.

The publication of the results is planned.