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

Action number: CA20129 MultIChem

Grantee name: Dorothea C. Hallier

Details of the STSM

Title: Irradiation of ssDNA-binding proteins with accelerated protons

Start and end date: 16/09/2024 to 04/10/2024

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 3 mg/mL. The protein was then shipped on dry ice to IPHC in Strasbourg, France.

Week 1: 16.09. - 21.09.2024
After the needed security and lab safety instructions, the sample preparations were initiated. At first, the procedure of producing high-concentrated native protein gels (with the help of glutaraldehyde) was performed with myoglobin, the well-studied model protein of the working group (see figure 1, left). Afterwards, the procedure was applied to G5P. Therefore, the concentration of G5P needed to be increased to about 20 mg/mL using ultracentrifugation for several hours. This was followed by a multi-step exchange of H2O with D2O in the concentrated solution using ultracentrifugation. Despite the numerous trials, the production of native gels was not successful. However, it was was possible to continue the project with dried samples (drop casted concentrated solution was dried on polypropylene films) and concentrated solution (drop casted concentrated solution on polypropylene films).
The production of concentrated solution and the successive exchange of H2O with D2O was continued during the whole STSM since only freshly produced solution was suitable for radiation experiments. This is due to the delicate structure of the protein and the required native and stable status of the protein for radiations. The quality of the produced samples was controlled with FT-IR spectroscopy.
It was possible to perform first irradiations at CIRCÉ radiation facility (24 MeV) with a diluted solution of the protein (1 mg/mL) with total doses of 5, 15, 40 and 100 Gy at low (0,14 Gy/s) and ultra-high (100 Gy/s) dose rates (see figure 1, right). The irradiated samples were stored at -20 °C until further analysis.

[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.

Week 2: 23.09. - 27.09.2024
The first irradiations at iCUBE (2 MeV) radiation facility were performed. First, a freshly prepared myoglobin gel was irradiated to be introduced into the procedure of irradiation with successive UV and FT-IR measurements after every applied dose. Then, freshly prepared G5P concentrated solutions were irradiated at room temperature and ambient pressure with FT-IR and UV surveillance for several hours (see figure 2, left). Unfortunately, the IR source of the FT-IR device broke during the first experiments but was replaced the next day, that'y why the irradiations were delayed and postponed to the third week.

Week 3: 30.09. - 04.10.2024
Further irradiations were performed at iCUBE (4 MeV) radiation facility. Firstly, a dried sample was irradiated under cryogenic conditions (25 K, under vacuum) under FT-IR surveillance. Then, a dried sample was irradiated at room temperature (25 °C, under vacuum) under FT-IR surveillance. Lastly, a dried samples was irradiated at ambient conditions, 25 °C, ambient pressure) under FT-IR and UV surveillance (see figure 2, right).
The applicant gave an oral presentation of her PhD project. The performed experiments and first results of the STSM were presented in the IPHC Radiochemistry group.

After the end of the STSM, the activity of the irradiated diluted solution (see Week 1) was examined using electromobility shift assay in the applicants home lab at Fraunhofer IZI-BB in Potsdam, Germany.
Due to the strong changes upon irradiation of the concentrated solutions, no activity measurements were performed. However, strong production of gasous compounds were observed in the irradiated sample (bubbles were visible). Due to the dried character of the dried samples, no activity measurements no activity measurements were performed.

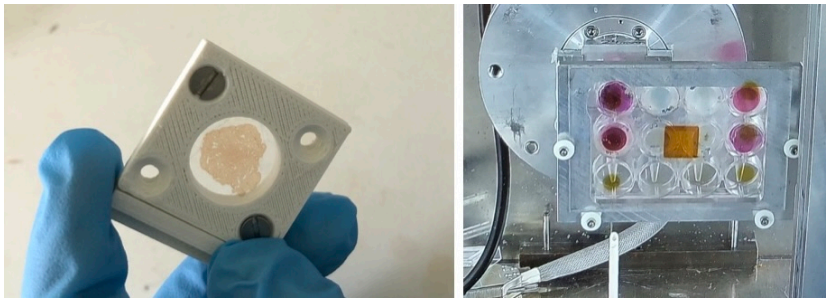

Figure 1: left: Freshly prepared myoglobin gel in sample holder. right: Irradiation of diluted samples (in eppendorf tubes (lowest row)) at CIRCÉ radiation facility 24 MeV

Figure 2: left: freshly prepared liquid G5P sample in sample holder. right: dried sample of G5P in sample holder

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 protons“ 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 accelerated protons.

All of the initially planned experiments were performed. In addition, irradiations at 24 MeV were performed:

- concentrated solutions were irradiated to mimc the biological conditions of proteins in cells (highly-concentrated protein in aqueous environment)
- dried samples were irradiated under cryogenic conditions to allow for a more specific analysis of the formed species upon irradiation. Furthermore, dried samples were irradiated to allow an improved comparison with already existing datasets measured under the influence of X-rays.
- diluted samples were irradiated at an additional radiation facility to be able to use different dose-rates and to allow an improved comparison with already existing datasets measured under the influence of X-rays

The secondary structure of G5P in different conditions upon irradiations was followed using FT-IR.
UV-visible spectra were measured to add-up to the above mentioned experiments to identify other species produced upon irradiation of the protein (such as carbon monoxide or other inorganic degradation products).
The experimental results are yet to be analyzed and understood in detail.

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, it is planned to perform HPLC-MS measurements of the diluted samples that were irradiated at CICRÉ 24 MeV radiation facility. Further measurements at the named beam line could be performed to be able to generate FT-IR data as well as circular dichroism data to be able to investigate on the development of the secondary structure of the diluted samples as well. Therefore, additional G5P protein in native

The publication of the results will follow.