Research Trip Summary Report

Task 2. Foreign mobility of WUST doctoral students

I. Data of the doctoral student

1.Full name: Maciej Lipok

2.Year of studies: 2

3. Educational discipline: Chemical Sciences

II. Foreign research trip (research visit)

1. Research institute in which the foreign research was implemented: University of Michigan, Ann Arbor, USA

2. Name and surname of the host person (mentor): professor Julie Biteen

3. Dates of the research trip: 14.03.2022-10.04.2022

4. Title and date of a seminar delivered during the research trip: “Optical properties of chiral heterostructures with gold nanoparticles”, Julie Biteen Lab group seminar, 17.03.2022

5. Description of work carried out during the research trip:

- Learning the theoretical principles behind single molecule localization microscopy (dSTORM technique)
- Learning how to use SMALL-LABS, an algorithm used to analyze the data collected using single molecule localization microscopy
- Amyloid sample preparation and optimization for single amyloid fibril imaging
- Imaging the single fibrils, stained with two amyloid-specific dyes: Thioflavin T and Congo Red
- Single gold nanoparticle sample preparation and optimization with different types (nanospheres, nanorods, nanobipyramids) and sizes (from 25nm to 95nm) of gold nanostructures
- Imaging and collecting scattering spectra from samples with single gold nanoparticles
- Preparing and optimizing the samples with gold nanoparticles bound to amyloid structures
- Imaging the amyloid-gold heterostructures using single molecule localization microscopy
- Data analysis using SMALL-LABS and self-written Python algorithms

6. Description of the main results obtained:

The main goal of the internship at University of Michigan was to find out if plasmonic nanoparticles in the vicinity of chiral amyloid structures can enhance their chiral field and probe it due

to behavior of amyloid-specific dye molecules near those structures. First days of the internship focused on learning the imaging system and theory behind it along with amyloid sample preparation. The systems was built of laser diodes with different emitting wavelengths, collimating optics, polarizers, liquid-crystal variable waveplate allowing to obtain left and right handed circular polarization of excitation laser, microscope mounted with an oil objective and CCD camera to collect the signal. All experiments were based on bovine insulin amyloids dissolved in MiliQ water with 25mM HCL and incubated for 18h in temperature elevated to 70°C. First set of measurements using the single molecule localization microscopy has shown that 1000 times dilution with pure MiliQ water is required to obtain sample with separated single fibrils. Moreover, drop casting the diluted amyloid solution on a plasma etched microscope slide already mounted in a system lead to best separation of amyloid structures. Two types of amyloid-specific dyes have been used to image amyloid fibrils – Thioflavin T (ThT) and Congo Red (CR). Dye concentration was adjusted to easily distinguish between the signal coming from amyloid fibrils and background, with acceptable signal-to-noise ratio: amyloids were stained with 20µM of ThT or CR before drop casting on a slide. Moreover, due to photobleaching of a fluorophore caused by a prolonged irradiation, small amounts of dyes (few drops of 2µM dye solution) have to be add every few collected movies. Due to fast photobleaching of Congo Red molecules all further experiments were based on amyloid fibrils stained with ThT only. Data analysis using SMALL-LABS and Python have confirmed that both dye molecules were binding to amyloid fibrils and could be used to image them using single molecule localization microscopy.

Next step of the internship was to prepare and image single gold nanoparticles. Four types of gold nanoparticles were prepared and measured: mini gold nanorods (25x10nm), gold nanobipyramids (50x20nm), gold nanorods (80x40nm) and gold nanospheres (diameter ~ 95nm). All nanoparticles were diluted 10-100 times with MiliQ water and drop casted on O_2 plasma etched microscope slides. Single gold nanoparticles were localized by scanning the slides in dark-field microscopy mode and collecting their scattering spectra using spectrophotometer aligned to collect the signal from microscope. Most promising results were collected from the 100 times diluted samples with bigger nanoparticles, showing clear scattering bands (one band for nanospheres and two bands for nanorods) correlating well with the extinction spectra collected using different spectrophotometer. Unfortunately, it was impossible to tell the difference between single and aggregated form of smaller nanoparticles (mini gold nanorods and gold nanobipyramids) because of their small scattering cross-sections. Thus, gold nanospheres and bigger gold nanorods were chosen for final experiments with amyloids.

During the last week different samples with amyloid-gold heterostructures were measured. Imaging showed that addition of sodium chloride, which enhances the binding of gold nanoparticles to amyloids, also causes a strong mutual aggregation of both insulin fibrils and gold nanoparticles resulting in big amyloid plaques with gold aggregates bound to them. However, further experiments have shown that the best strategy to create sample with single nanoparticles bound to single amyloid

fibrils is to first drop cast the solution with single fibrils on a slide already mounted in a microscope and then add nanoparticles. Several images with both single fibrils and aggregates with single nanospheres and nanorods bound to them were collected. Scattering spectra taken from the same nanoparticles have confirmed that nanoparticles have not aggregated.

In conclusion, most of the internship research program was successfully accomplished. Modification of the microscope system enabled circular polarization of excitation beam which was further used in all experiments. Sample preparation developed during the internship allowed to obtain and image both single insulin amyloid fibrils and their aggregates. Moreover, the correct dilution of nanoparticles resulted in sample with separated single nanospheres and nanorods, which was confirmed by their scattering spectra. In the end, both single amyloid fibrils and amyloid aggregates with single gold nanoparticles bound to them were prepared, and imaged using dSTORM technique. Unfortunately, due to limited time (4 weeks) only preliminary data analysis have been done, focused on visualizing amyloid fibrils to see if single molecule localization microscopy is an appropriate method to study them. More in-depth analysis and calculations are needed to show any chiral effects and answer if plasmonic nanoparticles can indeed enhance or sense the chirality of the structures and molecules around them.

7. Future collaborations (if applicable):

Data analysis of the results obtained during the internship, online meetings and discussion concerning the paper which will be based on the results obtained during the internship

8. Title and date of a seminar presenting the results of the trip delivered at Wroclaw University of Science and Technology after returning from the research trip: “Investigating chiral plasmonic response of gold nanoparticles bound to amyloid fibrils using single-molecule localization microscopy”, 20.04.2022

III. Doctoral student's signature

…….. (Date)

…….. (doctoral student's signature)

IV. Confirmation and information from the host

1. Confirmation of compliance of the information contained in the report: I CONFIRM / DO NOT CONFIRM. (*In justified cases, the confirmation of the host may be sent by e-mail to the Dean's Office of the Doctoral School email: interdocschool@pwr.edu.pl)*

2. Additional information and comments

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(Date) (signature(s) of Host)