**1. PHD PROJECT DESCRIPTION (4000 characters max., including the aims and work plan)**

**Project title:**

# *Studies on UV Radiation Impact on Proteins' Nanomechanic Using Single Molecule Force Spectroscopy*

## 1.1. Project goals

Single molecule studies are very challenging but possible these days, they are at the core of modern nanobiophysics. UV radiation can modify substantially biological matter. In this project we aim at providing an advanced tool to study UV effects in proteins on single molecule level.
We plan to work in the area of Single Molecule Force Spectroscpy (SMFS) and :

- To develop the state-of-the Magnetic Tweezer based set-up for measurements of UV-protein interactions.
- To explore possibilities of using AFM for studies of UV ipact on proteins, with main focus on SMFS.
- To gain understanding, at molecular level, on UV induced oxidation
- To gain better understanding of nanomechanics of selected proteins present in extracellular matrix, in particular at the synaptic cleft.

## 1.2. Outline

Ultraviolet radiation-induced protein damage has significant implications for many biological processes, including cellular signaling and DNA repair, and has been linked to various pathological conditions such as skin cancer and aging. UV radiation initiates the process of photooxidation, where the formation of reactive oxygen species (ROS) such as singlet oxygen and free radicals is observed. These ROS can damage/modify the protein structure by oxidizing amino acid residues, particularly tryptophan and tyrosine or can cause conformational changes through the breakage of disulfide bonds [1].

Forces are always linked with protein functions. Obviously forces play crucial roles in the "birth" and "death" of proteins, but recently a novel concept is emerging wherein mechanical domain unfolding can be vital factor in some biological processes. For instance, unfolding and extension of talin rod domains under force exposes binding sites for its vinculin ligand during mechano-transduction [2]. Forces are also linked with neuronal growth and functioning of synaptic cleft via force-mediated neurotransmitters release.

Single-molecule force spectroscopy (SMFS) has emerged as a useful approach for studying protein conformational changes (unfolding, stretching, collapsing, and refolding) in vitro. These techniques

can reproduce the force vector experienced by proteins in vivo along their end-to-end coordinate and can be used to evaluate nanomechanical properties, such as transition rates, diffusion coefficients, energy barriers, elastic, and molecular damping constants. A forced exposure of cryptic sites like disulfide bonds is also possible. Among various methods used for SMFS Atomic Force Microscopy (AFM) and Magnetic Tweezers (MT) show many advantages [3][4]. Both set-ups are available at the Department of Biophysics, Institute of Physics, NCU in Torun.

In the AFM force spectroscopy a molecule of interest is attached to a cantilever, which is then brought into contact with a surface or another molecule. As the cantilever is moved toward or away from the surface, the interaction force between the molecule and the surface or other molecule is measured, providing information on the mechanical properties and interactions of the molecule. By applying a series of increasing or decreasing forces to the molecule and monitoring the response of the cantilever, force spectroscopy can provide a detailed understanding of the molecular interactions and mechanical properties [5].

In MT force spectroscopy a molecule of interest is tethered between a surface and a magnetic bead, which is then subjected to a magnetic field gradient that applies a force to the molecule. The force can be adjusted by changing the strength of the magnetic field, allowing for precise control of the applied force. The position of the magnetic bead is monitored using optical microscopy and high speed camera, and changes in the position of the bead indicate changes in the length o of the tethered molecule. A major advantage of this method is the ability to maintain a constant force for a defined period of time, allowing a real time observation of protein refolding kinetics and its response to external factors or additives [6][7].

In this project such single molecule force spectroscopy methods will be applied to investigate the process UV oxidation of proteins. Through maintaining a constant unfolding force exposing disulfide bonds as they undergo UV induced oxidative damage a precise evaluation of changes in protein mechanical stability will be made. We will also make an attempt to study nanomechanics of proteins involved in neuronal extracellular matrix an/or present in neurons. In particular proteins over-expressed or linked to brain cancers will be studied (neuroligins and similar)

The project will be organized into the following key components:

1. The initial stages of this study will involve conducting a comprehensive literature review on prior research into single-molecule UV damage studies.

2. A literature review on neuronal protein studies using SMFS, especially MT is also envisaged.

3. The experimental setup will be developed, with a focus on optimizing the magnetic tweezers setup for the controlled application of UV to the sample. Appropriate proteins for use in the experiments will be identified and selected, and a detailed experimental protocol will be prepared.

4. Later stages of the study will involve the performance of preliminary experiments utilizing the chosen proteins with AFM and MT. These experiments will serve to optimize the experimental protocol and ensure that the study proceeds in an efficient and controlled manner. The primary experimental objective of this study is to subject the selected proteins to UV radiation in order to expose cryptic sites and evaluate the subsequent impact on their mechanical stability. We would like to observe the UV damage reversed by methionine sulfoxide reductase enzymes. We want to propose TM/AFM studies on nanomechanics of proteins present in ECM and neurons.

5. The last phase of this study will involve the composition of a comprehensive thesis, which will provide a detailed summary of the research findings obtained.

Literature:


[1] T. M. Nordlund i P. M. Hoffmann, *Quantitative Understanding of Biosystems: An Introduction to Biophysics, Second Edition*. CRC Press, 2019.

[2] I. Popa i R. Berkovich, „Mechanobiology: protein refolding under force", *Emerg. Top. Life Sci.*, t. 2, nr 5, s. 687–699, 2018.

[3] M. Mora, A. Stannard, i S. Garcia-Manyes, „The nanomechanics of individual proteins", *Chem. Soc. Rev.*, t. 49, nr 19, s. 6816–6832, 2020.

[4] J. Alegre-Cebollada, „Protein nanomechanics in biological context", *Biophys. Rev.*, t. 13, nr 4, s. 435–454, 2021.

[5] P. J. Bujalowski i A. F. Oberhauser, „Tracking unfolding and refolding reactions of single proteins using atomic force microscopy methods", *Methods*, t. 60, nr 2, s. 151–160, 2013.

[6] S. Gaire, R. Fabian Jr, I. Pegg, i A. Sarkar, „Magnetic tweezers: development and use in single-molecule research", *BioTechniques*, t. 72, nr 2, s. 65–72, 2021.

[7] R. Tapia-Rojo, A. Alonso-Caballero, C. L. Badilla, i J. M. Fernandez, „Identical Sequences, Different Behaviors: Protein Diversity Captured at the Single-Molecule Level", *bioRxiv*, s. 2021–02, 2021.


## 5. Required Initial Knowledge and Skills of the PhD Candidate:

The candidate for this Ph.D. project should have a background in physics (optics, exparimenatl physics), biophysics, some molecular biology background and or chemistry is a plus. Essential skills include:

- Eager to perform many long-lasting and difficult experiments
- Eager to construct new physical equipment (some engineer work)
- Familiar with computer-assisted data acquisition and analysis.
- Skils (or ready to learn) in protein samples handling, functionalization of surfaces or magnetic beads.

- Strong communication skills for presenting research findings and collaborating with interdisciplinary teams (English)
- Interest in biomedical research at molecular level.
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While expertise in specific areas mentioned in the project is not required, the candidate should demonstrate an eagerness to learn and engage in interdisciplinary research. The project will provide opportunities to develop knowledge and skills in modern experimental biophysics.

## 6. Expected Development of the PhD Candidate's Knowledge and Skills:

Throughout the project, the candidate will expand their knowledge and skills in:

- Mastering SMFS techniques
- Understanding UV protein interactions.
- Good knowledge of selected molecular process related to diseases ( impact of physics on biomedical research)
- Enhancing scientific communication through reports, presentations, and manuscript preparation
- Developing critical thinking, problem-solving abilities, and experimental design skills