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

Project title:

"Modeling of neuronal proteins localized in a synaptic cleft".

1.1. Project goals

Brain function is still to large extent a mystery. A major role of so abundant (>10**9) synaptic clefts between neurons can't be overestimated. Pre-and postsynaptic neuronal membranes contain numerous proteins which regulate signals transduction. Severe diseases are linked to such proteins malfunction. Recently, a major role of active cleavage of neuroligins by ADAM10 (**A Disintegrin and metalloproteinase domain-containing protein 10**,) metalloproteinase in fast glioma metastasis has been discovered. In this project we aim at understanding this and similar process on molecular level. So, the following goals are outlined:

- To develop the state-of-the art computer atomistic models of selected proteins present in neurons, i.a., ADAM10 and NLG3
- To gain understanding, at molecular level, how important metalloenzymes regulate activity of proteins present in synapses via extensive molecular dynamics simulations.
- Add understanding to molecular mechanism involved in brain cancer progression, in particular glioma.
- Explore new possibilities of optical control of ion channels by light-activated ligands/inhibitors (optogenetics)
- To propose a model for sNLG mitotic agent receptors.

1.2. Outline

The synaptic cleft is nanometer scale compartment in which periodically neurotransmitters are released. They are detected by receptors present in postsynaptic neurons. The proper dynamics and concentration of those neurotransmitter warrant good mental health and control of mood. Architecture of synapses is complex, nearly 200 pairs of different proteins are present in this region, among them neurexins, neuroligins, reelins, contactins etc. Proteins stick out of membrane into the cleft and sometimes they bridge neurons. Mutations in those proteins were

linked to disorders such as autism and schizophrenia. Some proteins are regulated by enzymes sheddases. They regulate density of proteins by cutting of some parts sticking into a synaptic space. We plan to model at least several such enzymes/target protein pairs, with a special focus on ADAM10 and NLG3. This system, if dysregulated, is probably responsible progress of a bad brain cancer type: glioma [1]. We studied nanomechanics (computationally and experimentally – AFM) of several types of synaptic proteins [2,3]. However, till now, there is no model of activity and action of ADAM type enzymes. In this project we will fill this gap.

The project will be organized into the following key components:

Literature Review and Model Development: Conducting a literature review on ADAM type enzymes, neuroligins and proteins related to synaptic clefts. Development aof all-atom models of key components, mainly ADAM10 and NLG3. based on available experimental data, structural information, and models already developed in our group.

Molecular Dynamics (MD) Simulations and Analysis: Performing MD simulations [4] to investigate the conformational changes, protein –protein interactions, enzymatic activity mechanism and hypothetical ligand binding to ASAM10 and other selected systems. Analysis of MD simulation trajectories to gain insights into the mechanisms underlying release of sNLG and proteinase activity. Some techniques from enhanced sampling methods and AI methods will be involved.

Validation and Integration: Validate the computational models and findings by comparing them with available experimental data. Desired outcome might be a proposal for new experiments testing mode of action of ADAM10 and/or similar compounds.

Search for possible selective control of ADAM10 inhibitors, presumably based on photo-activated compounds such as azobenzenes or similar (optogenetics track) [5].

The final stage will be the Thesis Writing: Summarize the research findings in a comprehensive monograph. Prepare for the defense by presenting the results and implications of the study to the scientific community.

1.3. **Work pl**an

- Mastering methods of molecular dynamics and architecture of neuronal cleft
- Studies on P2X7 and other purinergic receptors involved in cancer (glioma) [6].
- Construction of ADAM10 (and other sheddases) and neuroligins models
- Developing a computational scheme for ADAM10 mediated sNLGs release study (quantum-chemical calculations may be involved)

- Testing the approaches, reaching hundreds of nanosecond timescales of simulation or enhancing the slow conformational change of closing and opening the enzyme (enhanced sampling approaches).
- Search for light activated inhibitors of ADAM10 ad similar systems.

1.4. **Literature** *(max. 10 listed, as a suggestion for a PhD candidate)*

[1] Venkatesh H. S., Tam L. T., Woo P. J., Lennon J., Nagaraja S., Gillespie S. M., et al. (2017). Targeting neuronal activity-regulated neuroligin-3 dependency in high-grade glioma. *Nature* 549 533–537. 10.1038/nature24014; Dang NN, Li XB, Zhang M, Han C, Fan XY, Huang SH. NLGN3 Upregulates Expression of ADAM10 to Promote the Cleavage of NLGN3 *via* Activating the LYN Pathway in Human Gliomas. Front Cell Dev Biol. 2021 Aug 16;9:662763. doi: 10.3389/fcell.2021.662763.

[2] Nanomechanics of multidomain neuronal cell adhesion protein contactin revealed by single molecule AFM and SMD. K Mikulska-Ruminska, AJ Kulik, C Benadiba, I Bahar, G Dietler, W Nowak
Scientific reports 7 (1), 8852 (2018)

[3] Dynamics, nanomechanics and signal transduction in reelin repeats
K Mikulska-Ruminska, J Strzelecki, W Nowak
Scientific Reports 9 (1), 18974 (2019)

[4] Applications of computational methods to simulations of proteins dynamics
W Nowak; Handbook of Computational Chemistry, 1-43, Springer, (2016)

[5] Photo-switchable sulfonylureas binding to ATP-sensitive potassium channel reveal the mechanism of light-controlled insulin release, K Walczewska-Szewc, W Nowak, The Journal of Physical Chemistry B 125 (48), 13111-13121 (2022)

[6] Purinergic approach to effective glioma treatment with temozolomide reveals enhanced anti-cancer effects mediated by P2X7 receptor
B Szymczak, J Czarnecka, S Czach, W Nowak, K Roszek
Cellular Signalling 106, 110641 (2023)

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

The “optimum” candidate for this Ph.D. project should have a background in physics or chemistry. biophysics, some molecular biology, computational modeling. Essential skills include:

- Good command of Linux, some experience in programming (or eager to learn), particularly in Python, MATLAB/
- Strong communication skills for presenting research findings and collaborating with interdisciplinary teams
- 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 computational modeling, drug design, biophysics, and other related to theoretical enzymology and nanoscales.

6. Expected Development of the PhD Candidate's Knowledge and Skills:
Throughout the project, the candidate will expand their knowledge and skills in:

- Advanced computational modeling techniques
- Understanding enzyme-traget interactions role of modeling in medicine related research
- Enhancing scientific communication through reports, presentations, and manuscript preparation
- Developing critical thinking, problem-solving abilities, and experimental design skills
- By the end of the Ph.D. program, the candidate will have a strong foundation in computational modeling, expertise in studying membrane proteins, programming proficiency, and effective communication skills. These abilities will pave the way for a successful career in biophysics research and related disciplines.