nencki institute of experimental biology

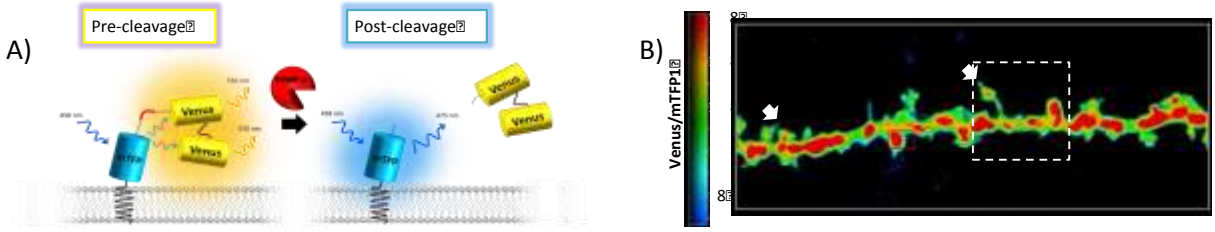
FRET-based biosensor of MMP-9 activity

Problem

Matrix metallopeptidase 9 (**MMP-9**), also known as gelatinase B, is an extracellularly operating enzyme involved in the modification of extracellular matrix. Recently the number of MMP-9 activity related publications has reached more than 700 publications every year indicating the growing interest in field of studies on this protein because of its anticipation in numerous biological functions. The most concern function of this protein is its major role in synaptic plasticity as the basis of **learning and long-term memory**. Moreover, aberrant MMP-9 activity also play an important role in the development of pathological conditions such as **epileptogenesis, arthritis, cerebral ischemia, atrial fibrillation or aortic aneurysms, ephemeral syndromes, fragile X syndrome, cancer, addictions and mental illness**. It is also involved in a number of physiological processes such as CNS development and plasticity, angiogenesis, cell survival, inflammation. The widely used substrate for testing MMP-9 activity is DQ™ gelatin (Life Technologies), although it has some major disadvantages as is not specific to MMP-9 only, but is also cleaved by coexisting MMP-2 and does not allow spatiotemporal monitoring of MMP-9 activity due to the lack of its specific localization. The lack of specificity is the most commonly raised objection against sensors used so far. Thus, it is a clear need of scientific world for a solution allowing specific and **real time monitoring** of **MMP-9 activity** at **subcellular resolution**.

Solution

We have developed a **BioMMP-9 ™ genetically encoded MMP-9 sensor** based on **FRET** (Förster/ Fluorescence Resonance Energy Transfer) mechanism: Sequence coding biosensor which when expressed in cells results in membrane attached protein expression and allows specific MMP-9 activity detection by fluorescent microscopy.



A: FRET based sensor mechanism: Pre-cleavage: donor and acceptor parts are close to each other, fluorescence emission is observed after exciting donor with light of appropriate wavelength. Post-cleavage: linker between parts is cut, no acceptor-emitted fluorescence is observed. **B: Dendrite of a hippocampal neuron in vitro**

<u>The most important advantages of our solutions:</u>

1. Allows the use of **natural mechanisms** to anchor the protein in the cell membrane and perform observation at subcellular, spatial resolution.
2. Enables the use of **fluorescent proteins** instead of synthetic dyes (**not toxic**).
3. Allows **greater temporal resolution** of observations of MMP-9 activity.
4. **Specific to MMP-9** (not cleaved by MMP-2), which specificity was confirmed in 2 research models: 1/ rat hippocampal neuronal cultures, 2/ MMP-9 knock out mice neuronal cultures.

Project Core Team

Michal Stawarski, PhD at the Nencki Institute of Experimental Biology, currently a postdock at Florida Atlantic University. Co-author of 4 research papers. **The principal investigator and inventor** of Genetically encoded FRET-based MMP-9 biosensor. Laureate of Award of Polish Academy of Science and Konorski Distinguished Work for the publication Stawarski et al., Genetically encoded FRET-based biosensor for imaging MMP-9 activity, Biomaterial 2014.

Jakub Wlodarczyk - head of **Laboratory of Cell Biophysics** at the Nencki Institute. PhD in physical science at University Warsaw, postdoctoral training at Max Planck Institute for Biophysical Chemistry. Author of 40 research articles (H-index 15) and **2 inventions** submitted for patent protection. Member of the Network of European Neuroscience Institute (ENI NET).

Leszek Kaczmarek is **professor of neurobiology** and head of the **Laboratory of Neurobiology** at the Nencki Institute. Chairs the Division of Biological and Agricultural Sciences, PAN and was the head of the Life Sciences Committee of the Council at the National Science Centre. He is an elected member of Polish Academy of Sciences, Academia Europaea, European Molecular Biology Organization (previously a member of the Council), and served on the Council of the International Society for Neurochemistry, and the Executive Committee of International Brain Research Organization, IBRO; was also the Vice-President of the European Molecular Biology Conference, EMBC. Visiting professor at the University of Catania (Italy), McGill University (Montreal, Canada), University of California, Los Angeles (USA), Institute of Optics and Photonics, Castelldefels, Spain. Author of over 200 research papers, cited over 8 500 times (**H-index 55**) and **3 patents and patent pendings**. Received several awards for his research achievements, including the most prestigious science award in Poland - a prize from the Foundation for Polish Science (2000).

About Nencki Institute

The Nencki Institute of Experimental Biology of the Polish Academy of Sciences is the largest non-university biological research center in Poland. High quality of research, excellent publication record, and strong international links place the Nencki among the leading biological institutions of Central and Eastern Europe. The main focus of Institute's research relates to novel therapies and diagnostic methods in diabetes, neurodegenerative diseases, neurological disorders, cancer and other diseases of modern civilization. The Nencki Institute also provide a wide range of services including preclinical trials, dermo-cosmetology studies, genetic engineering, transgenic animals production and biological imaging from electron microscopic to MRI levels. We appreciate the existing collaborations and **we are open to new cooperation with industrial entities to bring novel products to the pharmaceutical, biomedical and biotechnological market**.

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