**Applications of small molecule fluorescent sensor ZnDA:**

**Imaging of Zn2+ in lysosomes**

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Zinc is one of the most important metal elements in cell metabolism. It’s not surprising since zinc has been found to mediate more than 3000 proteins, affecting protein structure, catalytic properties and providing signal transmission. In view of the importance of zinc, a strict system for maintaining necessary concentration in the cell was evolved in the course of evolution. This system includes metal-binding proteins (metallothioneins) as well as multiple organelle-specific zinc transporters. Genetically determined disorders of this system lead to a wide range of diseases, from diabetes to neurological and cardiovascular disorders as well as various types of cancers. In addition to the genetic factor, dietary zinc deficiency contributes to the development of mentioned diseases. More than two billion people are believed to be affected worldwide. To develop a personalized zinc nutrition supply, study of zinc metabolism mechanisms is important. Lysosomes play an important role in maintaining homeostasis of Zn2+ in the cell as they are an integral part of the system of endocytosis - the exchange of matter with the environment. Since [Zn2+] level in lysosomes is poorly understood at the moment, and several reports published give different values, sometimes with a difference of 2-3 orders of magnitude, Zn2+ quantification in lysosomes using fluorescence microscopy method is the main subject of this research.

Fluorescence microscopy has proven to be a reliable and convenient method for studying biomolecules in the cell due to its high sensitivity and the ability to specifically label molecules of interest. The choice of the probe is one of the stumbling blocks in this method. Both fluorescent protein and small molecule probe approaches have been applied to that problem, but each has its advantages and drawbacks: fluorescent proteins are not stable towards acidic pH and oxidant compounds while the later are more robust, but suffer from lack of targetability to specific organelle and don’t remain in one compartment for long time imaging. The approach recently designed can overcome these challenges by combining the approaches above mentioned. In this method ZnDA, small molecule fluorophore [1], designed previously in the laboratory, is biding with HaloTag [2] labeling protein that is expressed in the cell and delivered to the organelle of study using corresponding signal sequence. Thus, targeting and probe retention for a long time in the organelle are achieved. In the previous study [3] the system was applied to several compartments such as nucleus, ER and mitochondria. In this study ZnDA probe was applied to acidic organelles. Colocalization of ZnDA probe with LysoTracker Red in lysosomes was performed followed by zinc quantification experiment. [Zn2+] level in lysosomes was assessed and compared with the level of other organelles. Additionally, using ZnDA probe, the data indicating zinc to be a substrate of TRPML1 channel was supported.

**References**

1. Kowada T., Watanabe T., Amagai Y., Liu R., Yamada M., Takahashi H., Matsui T., Inaba K., Mizukami S. **Quantitative imaging of labile Zn2+ in the Golgi apparatus using a localizable small-molecule fluorescent probe //** Cell Chem Biol. 2020. Vol. 27. P. 1521–1531.

2. Los G. V. et al. HaloTag: a novel protein labeling technology for cell imaging and protein analysis // ACS Chem Biol. 2008. Vol. 3. P. 373–382.

3. Liu R., Kowada T., Du Y., Amagai Y., Matsui T., Inaba K., Mizukami S. Organelle-level labile Zn2+ mapping based on targetable fluorescent sensors // ACS Sens. 2022. Vol. 7. P. 748–757.