

Warsaw, 04/09/2025

Author of the doctoral dissertation: **Aneta Magiera, M. Sc.**

Supervisor: **Robert Holyst, D. Sc.**

Auxiliary supervisor or Supervisor: **Karina Kwapiszewska, Ph.D.** (* optional only for the new proceedings for the award of a doctoral degree)

Second supervisor (optional only for the registration and conferment procedure for a doctoral degree): not applicable

Co-supervisor (optional only for the registration and conferment procedure for a doctoral degree): not applicable

Title of the doctoral dissertation: ***“Study of the internalization of chemical and biological molecules into living cells”***

Abstract

The penetration of any molecule into human cells, known as internalization, depends on the properties of that molecule, such as size, hydrophobicity, or electrical charge. When considering a molecule of therapeutic importance, an existing or promising drug, its internalization may or may not be a desirable process. Its mechanism of action determines this - some molecules bind to a protein inside the cell. Others, in turn, interact with a ligand found extracellularly. So, these two examples are desirable internalization and non-internalization, respectively. The phenomenon of drug penetration into the cell is particularly relevant in terms of the vigorously developed and tested targeted therapies. Such approaches are designed to reduce the side effects of standard cancer treatments by targeting the drug precisely to a selected element of the cancer cell. Thus, determining whether and to what extent a molecule with therapeutic potential penetrates cells appears to be the initial step in its testing in clinical applications.

In the present study, the internalization of various biological and chemical molecules (including drugs, dinucleotides, proteins, and a selected dye) into the interior of living, single cancer cells was evaluated. Fluorescence correlation spectroscopy was utilized as the primary research method. Precise, quantitative analysis was possible through the application of a length-scale-dependent nanoviscosity model of the cell interior. As a companion qualitative technique, confocal/FLIM imaging was used. The results showed that the adopted research approach makes it possible to determine what happens to a fluorescently labeled molecule intracellularly and what interactions this molecule undergoes.

Using the olaparib drug as an example, it was shown that the degree of penetration of this compound into cancer cells depends on the amount of its molecular target, the PARP1 protein. The FCS technique was also used to monitor the endocytosis process of the bevacizumab drug. As shown, this extracellularly acting compound is taken up by cells through constitutive endocytosis. However, it is not released intracellularly from endosomes into the cytoplasm. It was also

investigated whether selected therapeutic dinucleotides enter cells and are present in cells in a freely diffusing form. In turn, for ferritin and hemoglobin proteins, it was determined which oligomeric forms predominate inside cells. The studies also established that the YO-PRO-1 dye selectively stains tRNA and rRNA molecules in living cells. The example of the PRMT1 protein confirmed the applicability of the FCS technique for evaluating the intracellular distribution of a compound across different cellular compartments

Finally, the application of osmotic pressure changes to deliver various compounds (that are not spontaneously taken up by cells) to human cells was investigated. It was shown that the use of polymer in the regime of entanglement of polymer chains is a parameter that determines the success of the whole process. Based on the obtained results, a mechanism for introducing molecules into human cells by osmotic shock was proposed.