Warsaw, **26TH March 2025**

Author of the doctoral dissertation: **"BIOPHYSICAL SYMPTOMS OF CELLULAR STRESS"**

Supervisor: **ROBERT HOLYST, Ph.D., D. Sc.**

Auxiliary supervisor or Supervisor: **KARINA KWAPISZEWSKA, Ph.D.**

Second supervisor (optional only for the registration and conferment procedure for a doctoral degree):

.....

Co-supervisor (optional only for the registration and conferment procedure for a doctoral degree):

.....

Title of the doctoral dissertation: **BIOPHYSICAL SYMPTOMS OF CELLULAR STRESS**

Abstract

The intracellular molecular organisation is of utmost importance for a eukaryotic cell to stay alive. This organisation leads to the formation of a complex fluid called cell cytoplasm, where major biochemical processes occur. This drives functions like cell growth, division, and movement. Stress causes disturbances in the intracellular organisation. Cell survival during stress relies on changes in physical and biological properties. These changes in the complex, crowded fluid reveal important information about regulating major cellular processes and their role in cell survival. Limited information is available on biophysical processes during cellular stress. Fluorescence-based methods such as Fluorescence correlation spectroscopy (FCS) and Fluorescence lifetime imaging (FLIM) are excellent at measuring the diffusion dynamics, molecular interactions, and fluorescence lifetimes of solutes in low concentrations (<1 μM) and small volumes (<1 μl), making them ideal analytical tools for this study.

Stress affects every organism on the planet in different forms. Whether it's nutrient limitations, changes in oxidation/reduction states, increases in cytotoxic molecules, or changes in water content. The ways are unlimited. I picked two stress factors that affect all the organisms, irrespective of their size or complexity – cellular starvation and mitochondrial stress. I studied the effects of external stress on the biophysical properties of cancer cells. This thesis will describe a crucial survival technique employed by every living species on the planet – Stress-responsive behaviour.

In the **cellular starvation** chapter, I identify trends in intracellular molecular crowding and water efflux from cell cytoplasm and nucleus during prolonged starvation. FCS measurements reveal the effects of molecular crowding on green fluorescent protein (GFP) and define the changes in movement by measuring its diffusion coefficients. Without this intracellular motion/transport of molecules from one place to another, cells can't function. The chapter highlights some key points, such as compartmentalised cellular response to starvation stress, explicitly focusing on alterations in diffusion coefficients and energy conservation mechanisms. As an extension of these results, the effects of gene knockout of an anion channel are explored on diffusion of GFP and cell volume homeostasis in HeLa knockout cells. The gene knockout was created using CRISPR-CAS9 gene editing tools, and FCS was used to study intracellular diffusion.

In the **cellular autofluorescence** chapter, cell response to "induced cell death" via mitochondrial stress is measured. Measuring these changes is important to understanding cell response to stress before cell death. Using FLIM, FCS, and HPLC, I focus on the enhanced autofluorescence response of cancer cells to the apoptosis-inducing drug - staurosporine at two different concentrations.

The cellular ATP depletion chapter describes the limitations of ATP sensors, with a significant focus on a newly discovered chemical ATP sensor. Since starvation was a necessary stress factor in this study, alteration in ATP levels was considered as an interlinked phenomenon.