The concepts of life and death accompany people from the beginning of history. Defining these concepts, however, is difficult, because there are no clear premises separating these two states, and there are even organisms that do not fit into most definitions of living systems (eg viruses). Similarly, it is problematic to determine the unambiguous boundary between life and death at the level of individual cells. In this project, we present the hypothesis that the **limiting factor, determining cell death is inhibition of intracellular transport** - and hence – majority of biochemical reactions

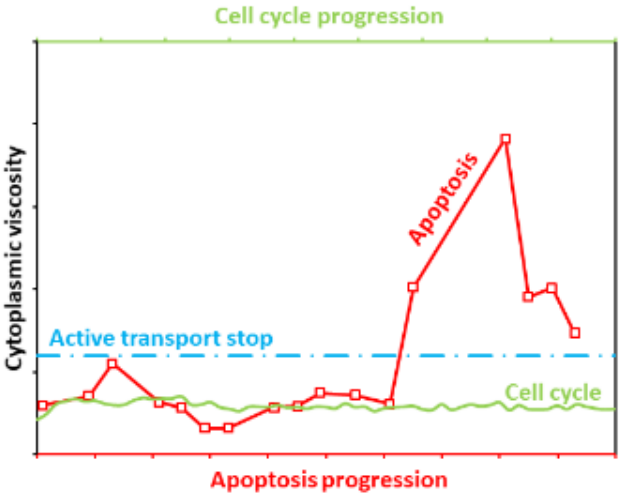

Figure: Values of cytoplasmic viscosity (probed by GFP protein) in different cellular states. Green curve (cell cycle) presents viscosity during undisturbed life cycle of human cell. Blue line (active transport stop) is a level, above which kinesin-1 is stooped – a molecular motor responsible for active transport in a cell. Red curve (apoptosis) presents changes of cytoplasmic viscosity during programmed cell death – apoptosis (preliminary results of the authors of the project).

We propose a novel approach to the study of intracellular processes by describing the basic physical properties of the cell. In order for the life processes (chemical reactions) to take place, it is necessary to move the molecules to close proximity. The process determining the movement of biomolecules is diffusion – the spontaneous movement of particles. On the other hand, viscosity is a property of the medium (cytoplasm), which inhibits diffusion. The Soft Matter Research Group at the Institute of Physical Chemistry of the PAS specializes in the study of the viscosity of the cellular interior at the nanoscale (1-100 nm). So far research of the Group allowed to observe the stability of values of cytoplasmic nanoviscosity in various cell types and during the entire cell cycle – it is probably attributed to an unknown mechanism maintaining this value at a certain optimal level (about 2-4 viscosities of water under the same conditions, measured for small proteins <40kDa). In addition, we observed that molecular motors - proteins responsible for active transport in the cell - are stopped with a slight increase in nanoviscosity (up to 6 viscosities of water). This led to the idea that the **increase in cytoplasmic nanoviscosity means the inhibition of life processes** and, consequently, death. We conducted **preliminary studies that showed that programmed cell death (apoptosis) is accompanied by an increase of nanoviscosity** >10 viscosities of water. In this project, we want to conduct systematic research of the observed phenomenon.

In the course of the project, the cytoplasmic nanoviscosity will be examined during programmed cell death: apoptosis and necroptosis. Cells of different ages and origins will be subjected to these processes in order to observe phenomena universal for various types of tissues. We will study nanoviscosity at various length scales (1-100 nm) and the movement of particles at different time scales (from microseconds to seconds). We will apply correlation techniques: fluorescence correlation spectroscopy (FCS) and raster image correlation spectroscopy (RICS), which are successfully developed in our laboratory. In addition, we will use a new, complementary to FCS, analysis of slow movements of biomolecules - BiWEC (developed from scratch in our group). The result of these works will be a systematic analysis of the mobility changes of intracellular components at all length scales during apoptosis and necroptosis. We will follow the movement of known fluorescent probes as well as native autofluorescence components. This second strategy - the use of fluorescence naturally occurring in cells - may result in **a new technique for determining cell viability, which does not require the use of a dye (a label-free assay)**. Such techniques are sought after to simplify procedures and reduce costs and waste in toxicological studies.