## Project 3.6 Horizon for Excellence in messenger RNA applications in immunoOncology: Quantitative analysis of mRNA in cells

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## **Background:**

Most current experimental methods for real-time measurements in single cells provide only qualitative and semiquantitative insights into the molecular mechanisms of interest. More detailed data can be obtained in highthroughput assays (biochemical assays with cell extracts) or from studies on fixed cells (various labeling procedures). This technological gap prevents the understanding of the kinetics of subcellular processes in particular. This gap can be filled by a technique which uses Fluorescence Correlation Spectroscopy (FCS), a method that allows a determination of the number and the diffusion time of single molecules in a sample. This well-known technique has found limited application in living cells due to the difficulty in interpreting data regarding "diffusion time" in particular. Robert Holyst's group has conducted research for over 10 years to develop an empirical model linking the size of molecules and the hydrodynamic resistance they encounter in complex fluids, including the cytoplasm of cells and the interior of cell nucleus. This model and the resulting equation allows direct calculation of the size of diffusing molecules inside the cell. This not only allows us to determine the number of moving molecules, but also their average size and their number.

## Aim:

The goal of the project will be application of advanced fluorescence techniques to answer fundamental questions about interactions of exogenous mRNA in the cellular interior. To this end, the original, proprietary method for real-time measurements of molecular interaction parameters in living cells will be used. This method is based on fluorescence correlation spectroscopy (FCS), supported by quantitative fluorescence microscopy and fluorescence lifetime imaging (FLIM).

## **Requirements:**

- Education: Master degree of Chemistry or related discipline,
- achieved before the work in project starts,
- good knowledge of English,
- CV and cover letter,
- participation in recruitment for Warsaw 4PhD is obligatory with simultaneous application of a candidate for recruitment for the position of a PhD student-scholarship in the competition WIB (Wirtualny Instytut Badawczy) which will be realized in IChF PAN. More details: kkwapiszewska@ichf.edu.pl,
- strong motivation and commitment,
- knowledge of the FCS technique (Fluorescence Correlation Spectroscopy) and the basics of microfluidic techniques will be a welcome advantage.