The information about processes inside the cells , which are caused by pH changes or which can induce pH fluctuation, are important not only in terms of understanding the mechanisms which rule the cell biology, but also for biotechnology purposes and molecular diagnostics. For example, dysregulated pH is known to be an adaptive feature of most cancers, regardless of their tissue origin or genetic background. Therefore, the measurements of pH within tumors, both intra- and extracellular, are necessary and should be done with non-destructive and highly sensitive techniques. The ability to control cell's proliferactive activity by means of pH sensors can be helpful in designing anticancer drugs. Recently, the pH sensors based on i-motif folding have became an attractive alternative to the conventional pH-sensitive indicators mainly due to narrower transition window and ability to tune their working range.

The DNA tetraplex structure called 'i-motif' is formed by C-rich oligomers and must be proceeded by formation of hemiprotonated cytosine-cytosine (CH^+–C) base pairs. Due to the conformational sensitivity of C-rich oligonucleotides to pH changes, examples of nanoswitches and biosensors based on the i-motif structures have been reported.

The principal goal of our research is the development and optimization of a new class fluorescent pH sensors based on molecular beacons containing C-rich sequences able to fold into i-motifs and to define their working range. In our first approach, the proposed system would be labeled by pyrene tags at the both ends. Next, we would like to attach pyrene moiety to one of oligonucleotide's ends or to nucleobase at loop position of i-motif part. We assumed that the formation of the i- motif will contribute to the effective interaction between the fluorophores (pyrene / pyrene or pyrene / nucleobase), resulting in the appearance of excimer or exciplex emission (EMS) sensitive to pH changes in physiological range (between pH 7.5 and pH 5.5) and with a good response resolution (even 0.1 pH unit).

The simplicity of these light switch systems is an indisputable advantage. The significance of the proposed research is that the pyrene-functionalized molecular sensor would provide a better method of detecting changes in pH than is offered by the dyes that are currently used.

We are planning to obtain set of pyrene-functionalized molecular beacons containing C-rich sequences able to fold into i-motifs and perform their spectral characterization by using UV-vis, CD, steady-state as well as time-resolved fluorescence spectroscopy techniques. The photophysical properties and photochemical stability of the fluorescent systems will be study in the various pH solution These experiments will help us to select the proper C-rich DNA sequences in order to obtain the best fluorescence answer for pH changes.

An important goal is to expand project to design i-motif based probes that would be nuclease resistance (2'OMeRNA analogs) that the cell biologists who eventually use these sensors would get the stable and reliable analytical signal. The biochemical studies of obtained molecular beacons based on unmodified as well as modified DNA sequence are planned and include testing degradation ability of our probes in test tube with blood serum, purified nuclease enzymes or in cell lysate. Finally, we would like to evaluate the utility of our system as intra- and extracellular pH sensor. The practical application of our probes can be performed by means of confocal laser scanning microscope which is suitable for investigation of spatial and temporal pH gradients in living cell.

We believe that our systematic research study will give as a final result an excellent optimized fluorescent molecular sensor able to serve as an exquisitely sensitive pH indicator. As we said before, the measurements of pH within tumors, both intra- and extracellular, are necessary and should be done with non-destructive and highly sensitive techniques. Additionally, the investigations of biophysical properties of fluorescent molecular beacons containing C-rich sequences able to fold into i-motifs based on unmodified as well as modified DNA sequence may contribute to clarify and understanding the biological role of these kinds of tetraplexes.