Every day, our organisms are exposed to numerous external and/or environmental factors that may influence our functioning, health, and our well-being. Our genome itself is constantly being modified as a part of normal development or in response to extrinsic/environmental factors. The modifications of DNA occurring naturally in mammals that can modify functionality of DNA segments and influence our genotype without changing the primary nucleotide sequences are known under the term *epigenetic modifications of DNA*. Some of these modifications are essential for many biochemical processing occurring normally in our body, however, they are also associated with various human diseases, for instance cancers, insulin resistance and obesity, neurodegenerative Alzheimer's and Parkinson's diseases, and more.

DNA methylation is known as one of the most important epigenetic modification processes occurring naturally in mammals. This process affects structural and mechanical properties of DNA strands and in consequence, it determines an interaction between DNA and other molecules like proteins, peptides, and drugs. On the other hand, alternations of DNA methylation are considered as a hallmark of carcinogenesis. For example, the abnormal patterns of DNA methylation occur at early stages in neoplastic regions of pancreas. Understanding of the cumulative role of epigenetics mechanisms in the pathogenesis of the pancreatic cancer is beneficial for a development of successful treatment regimes.

The methylation of DNA is known to affect the structural properties of DNA helix. However, due to the lack of an appropriate experimental techniques, the structural properties and functionalities related to DNA methylation, heterogeneous at the nanoscale, are still not fully explored. Methodological and instrumental limitations prevented or largely prohibited full understanding of local properties of modified DNA. Therefore, I propose to involve highly sensitive techniques providing local chemical information at nanoscale, specifically, at the site of individual modification of DNA strand. Recent progress of nanospectroscopic techniques has opened the possibility of studying chemical structure at the level of single molecules. Nanospectroscpy combines the chemical sensitivity of conventional spectroscopy (Raman or infrared spectroscopy) with the resolution of Scanning Probe Microscopy (SPM) techniques. In this project, I propose to incorporate Tip-enhanced Raman spectroscopy (TERS) as the most sensitive technique enabling exploring the structure of individual DNA helix and its conformational properties, Fourier Transform Infrared nanospectroscopy (nano-FTIR) as a complementary method, and SERS (Surface-Enhanced Raman Spectroscopy) as a reference bulk method. The structural rearrangements at the level of single molecules will be studied in a systematic manner, starting from commercially available methylated DNA, through the DNA strands and chromatin extracted from cultivated cells of pancreatic cancer to pancreatic cancer tissues. Additionally, the influence of selected anti-cancer drug treatment on pancreatic cancer cells will be explored to gain insights into local structural rearrangements of DNA exposed to anti-cancer treatment regimes. Moreover, the advanced methods of controlled nanoengineering exploited in fabrication of biocompatible plasmonic nanoprobes applied during nanospectroscopic investigations (eg. thin layer deposition and electron beam nanolithography, FIB/SEM nanostructuring, and laser beam processing) will ensure the highest possible sensitivity of applied methods, which was limited so far. To support the experimental findings of controlled preparation of sensitive bioprobes, I propose to incorporate theoretical simulations (using finite-element simulation packets COMSOL) that will allow to optimize their geometry and composition.

The results of this project will allow to gain the essential knowledge about the structural aspects related to epigenetic modifications of individual DNA strands at nanoscale. Thus, it will be possible to explore the role of methylation patterns in pathogenesis of the pancreatic cancer. Moreover, the project will provide the universal methodology to study epigenetic DNA modification process locally, either at the level of individual DNA molecules, or even at specific sites of selected genomic sequences. Such a concept of ultimate characterization of the molecules of life, such as DNA, will shed new light on our understanding of biological processes, which are crucial for life.