DESCRIPTION FOR THE GENERAL PUBLIC

DNA in single cell might be damaged even tens of thousands times per one day. As a result of damage various types of abnormal modifications of DNA structure could be formed such as chemical modifications of bases, pyrimidine dimers formation through cross-links (covalent bonds between bases). DNA backbone might also be interrupted leading to single strand breaks (SSBs) and double strand breaks (DSBs). DSBs are the most dangerous for cells. Unrepaired DSBs may cause even cellular death. Cells developed several complex mechanisms of DNA damage repair.
Proteins involved in DNA damage repair are well known but many fundamental aspects of the DNA repair process still remain elusive. Due to methodological limitations it was impossible to directly measure structures, compositions and orientations of protein complexes as well as structural modifications of DNA itself. DNA structure may change locally upon damage induction and interaction with repair protein. This change is strictly related with DNA reactivity/susceptibility to bind particular repair protein. DNA together with proteins called histones form chromatin. The chromatin integrity strictly affects local conformational transition of DNA.

Here we would like to address fundamental questions related to an influence of DNA conformation and also chromatin integrity on the susceptibility to DNA damage formation and repair. This will be done by application of tip-enhanced Raman (TER) spectroscopy and mapping supported with other analytical techniques to damaged DNA/chromatin and DNA/chromatin-repair protein complexes. TERS is a combination of Atomic Force Microscopy, which will allow for DNA and repair proteins imaging and Raman spectroscopy for chemical analysis. With TERS one can study chemical structure and composition with nano-metric spatial resolution. In this project we will directly follow DNA conformation upon damage induction with chemotherapeutic drug bleomycin and interaction with repair proteins such as DNA ligase IV and MutS.

In addition, we are also planning an application of fluorescence microscopy and infrared spectroscopy to detect DNA damage in cells and to study cellular response to induced lesions at molecular level respectively. We expect to accurately visualize chromatin fragmentation of and detect the phosphorylation of histone proteins associated with the repair of double strand breaks with fluorescence microscopy. Infrared spectroscopy will be applied in studies of living cells, isolated nuclei and chromosomes, in order to detect changes in the chemical composition and structure of bio-molecules in response to DNA damage induced by bleomycin. In cells treated with bleomycin and cell nuclei and chromosomes isolated from them, we expect to observe an increase of protein expression and DNA conformational change associated with the repair process.

The proposed project includes complementary studies at various levels of chromatin integrity: single DNA strands and chromatin fibers, as well as chromosomes, cellular nuclei and cells. Appropriate analytical techniques and imaging methods were carefully selected to each of the above-mentioned samples to obtain complementary information of the role of DNA conformational changes in DNA damage formation and repair.

The project requires significant optimization of experimental methods, in particular TERS technique for measurements in liquids. Optimised methodology could possibly be applied by chemists, biologists and biophysicists for investigation of many delicate biological systems such as aggregating neurodegenerative peptides, chemotherapeutic drugs binding to DNA or formation of domains in thin lipid layers under the introduction of membrane proteins.