DESCRIPTION FOR THE GENERAL PUBLIC

Each cell has to continuously repair DNA damage. Even tens of thousands of DNA lesions occur in one cell per day. Particularly noteworthy is oxidative damage (caused by reactive oxygen species, mainly hydroxyl radicals) resulting from oxidative stress, which is important in many civilization diseases such as atherosclerosis, Alzheimer's disease, or Parkinson's disease. Various types of abnormal modifications of DNA structure, the so-called DNA damage, could be formed: modifications of DNA bases, pyrimidine dimers formation, through covalent bonds between bases called cross-links. Damaging factors may also interrupt one or both DNA strands leading to single strand breaks (SSBs) and double strand breaks (DSBs), respectively. DSBs are extremely dangerous. Unrepaired DSBs may cause mutations, chromosomal aberrations and even cellular death. Therefore, cells developed several very complex mechanisms to repair the DNA damage. Proteins responsible for the repair of DNA damage are well known. However, many fundamental aspects of DNA – proteins interaction upon DNA repair process still require further research. Methodological limitations prevented or largely prohibited direct measurements that would provide information about structure and composition of DNA-protein complexes as well as the molecular modifications of DNA itself. DNA structure may be locally modified (local conformational change) upon strand break or interaction with repair proteins. This change determines DNA reactivity with a particular repair protein.
Here, a deep investigation into the influence of DNA conformation and chromatin integrity on the susceptibility to DNA damage formation and the efficacy of its repair is planned. To verify local molecular structure, an application of extremely sensitive analytical technique called tip-enhanced Raman (TER) spectroscopy and also TER spectral mapping supported with other analytical techniques to damaged DNA/chromatin and DNA/chromatin-repair protein complexes is planned. TERS is a combination of Atomic Force Microscopy, allowing an imaging of the studied molecules (here DNA and repair proteins) and Raman spectroscopy enabling the analysis of chemical structure. TERS provides information about chemical structure and composition with nano-metric spatial resolution. Precisely, I am planning to follow DNA conformational modifications upon oxidative damage induction with hydroxyl radical and interaction with repair proteins such as DNA ligase IV and MutS.

Additionally, infrared and Raman spectroscopies will be applied to detect DNA damage in cells as well as a hallmark of cellular response to induced DNA oxidative damage. Infrared spectroscopy will be applied in studies of living cells and isolated nuclei. Individual chromosomes, nuclei and cells will be also mapped by Raman microspectroscopic imaging. In cells treated with H_2O_2 as well as nuclei and chromosomes isolated from them, we expect to observe an increase of protein expression and DNA conformational change associated with the DNA repair process.

Summarising, this 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 related to the role of DNA conformational changes in DNA damage formation and repair. An integral part of this project is an application od multivariate data analysis for simultaneous treatment of all the acquired data for comprehensive comparison of all micro- and nanospectroscopic markers (characteristic marker bands) associated to DNA damage and repair.

This Project involves significant development of experimental methods, in particular an implementation of TERS technique for measurements in liquid. This will be beneficial for other researchers, who will apply the optimised methodology in deep investigation into many biologically significant systems.