INSTITUTE OF BIOCHEMISTRY AND BIOPHYSICS
POLISH ACADEMY OF SCIENCES

1. **Research Unit:** Laboratory of Molecular Basis of Biological Activity
2. **Supervisor:** dr hab. Agnieszka M. Maciejewska
3. **Supervisor (email):** agniesza@ibb.waw.pl
4. **Project title (English):** Repair of ethenoadducts to DNA and RNA bases by human AlkB family dioxygenases - substrate specificity, thermodynamics and molecular mechanism of action
5. **Project title (Polish):** Naprawa etenoadduktów do zasad DNA i RNA przez ludzkie dioksygenazy z rodziny AlkB - specyficzność substratowa, termodynamika i molekularny mechanizm działania
6. **Description of the project (up to 500 words):**

The AlkB-type dioxygenases are repair enzymes which remove alkyl- and exocyclic adducts from nucleic bases *via* a newly discovered oxidative mechanism restoring native DNA structure. It was found that they also repair methylated RNA lesions. Exocyclic adducts occur as an effect of DNA reaction with endogenous products of lipid peroxidation as well as with environmental carcinogens. Studies on the biochemistry of the AlkB family have lasted only several years and there is still a need for inventive discoveries. Recently, we have confirmed that *E. coli* AlkB repairs 3,N^4-ethenocytosine and 1,N^6-ethenoadenine in DNA and demonstrated that AlkB removes the additional saturated exocyclic ring from 3,N^4-α-hydroxyethanocytosine (HEC) and 3,N^4-α-hydroxypropanocytosine (HPC). Our *in vitro* observations allowed us formulating the hypothesis that AlkB preferably recognizes and repairs protonated (cationic) form of substrates. On the basis of structures accessible in PDB we have indicated aminoacids, which are most possibly involved in substrate recognition.

Main aims:

- The comprehensive study of substrate specificity of human ABH 2-3 dioxygenases against selected exocyclic adducts to DNA and RNA bases.
- *in vivo* verification of RNA lesions repair importance.
- Elucidation of the molecular mechanism of action of the dioxygenases.
- Explanation of the role of substrate protonation during its binding and repair in ABH 2-3 active centre, and the exact mechanism of substrate identification.
- Identification of the repair reactions intermediates that may constitute an essential thermodynamic barrier in post-processivity.
- Verification by site-specific mutagenesis of the role of negatively charged aminoacid in the dioxygenases active centre.
- *in silico* and experimental clarification whether ABH3 differs from all other AlkB homologues or the differences observed in PDB record of ABH3 results from Leu177 modification.

High similarity between AlkB-type dioxygenases allows generalization of the conclusions to all the AlkB family members.

Within the project:

- Using the HPLC technique we will determine the optimal conditions for the repair of exocyclic adducts to DNA and RNA by ABH 2-3 dioxygenases and by their rationally mutated forms.
- We will compare the *in vivo* level of methyl- and ethenoadducts do RNA bases formed spontaneously and induced by MMS and CAA treatment and check if AlkB-like dioxygenases take part in their repair.
- The effect of protonation of the substrate on its interaction with the catalytic center of ABH 2-3 will be analyzed using site-directed mutagenesis to replace two non-catalytic amino acids proximal to the active center of the protein.

• The newly observed intermediates of repair reaction of HEC, HPC and HPA will be identified by mass spectrometry. This will allow determining the exact mechanism of their repair.
• The thermodynamic parameters will be determined using fluorimetry, microscale thermophoresis (MST), circular dichroism and isothermal titration calorimetry (ITC).
• Kinetic parameters will be obtained by a combination of the time evolution of series of MS spectra and by HPLC analyses of the reaction mixture.
• Structural information will be obtained with the aid of molecular modeling using all the available structures of AlkB proteins.

7. **References related to conducted /planned research (maximum 3)**:

1. 1,N^6-alpha-hydroxypropanoadenine, the acrolein adduct to adenine, is a substrate for AlkB dioxygenase Dylewska Malgorzata; Kusmierek Jaroslaw T.; Pilzys Tomasz; Poznański Jarosław; Maciejewska Agnieszka M. *Biochem. J.* 474 pp. 1837-1852 DOI: 10.1042/BCJ20161008
2. AlkB Dioxygenase Preferentially Repairs Protonated Substrates. SPECIFICITY AGAINST EXOCYCLIC ADDUCTS AND MOLECULAR MECHANISM OF ACTION
Maciejewska Agnieszka M.; Poznański Jarosław; Kaczmarska Zuzanna; Krowisz Beata; Nieminuszczy Jadwiga; Polkowska-Nowakowska Agnieszka; Grzesiuk Elżbieta; Kuśmierek, Jarosław T. *J. Biol. Chem.* 288 pp. 432-441 DOI: 10.1074/jbc.M112.353342

8. **Scholarship amount (net):** 3000 PLN for mid-term evaluation, after mid-term evaluation, change to 57% professor's remuneration (currently it would be 3242 PLN net).