Proteins are the major and most divers constituent of all cells, playing a key role in living systems. Their function and activity is tightly controlled through multiple mechanisms, among which post-translational modifications (PTMs) appear to be essential. PTMs mainly refer to covalent additions of some chemical groups to side chains of amino acids building a protein. Such modifications are introduced by enzymes - dedicated proteins able to catalyze specific chemical reactions. Though the role and enzymology of most PTMs have been fully elucidated, yet there is very little knowledge and little research of several protein modifications. Recent studies indicate that human SETD3 enzyme plausibly catalyzes N-methylation of numerous intracellular proteins, though the biochemistry of this reaction and biological role of this enzyme remain unclear.

Taking into account that post-translational modifications are involved in regulating almost all cellular processes and perturbation in activity of PTMs-introducing enzymes frequently disturbs cell physiology leading to various diseases, including cancer, the aim of the present project is to:

1) produce large quantities of "synthetic", recombinant human SETD3 protein,
2) generate mammalian cells devoid of the enzyme activity that will be employed in searching for its novel protein substrates,
3) produce recombinant forms of identified substrates and confirm their modification by the investigated enzyme *in vitro*,
4) identify the mechanism determining substrate specificity of SETD3 enzyme.

To achieve our goals, human recombinant SETD3 *N*-methyltransferase will be produced in mammalian cells (COS7), purified to the homogeneity and shown enzymatically active, employing radiochemical assays. CRISPR/Cas9 system will be used to engineer human cell lines showing no activity of the enzyme studied, while SETD3 knock-out cells will serve as a convenient source of its novel intracellular substrates. Finally, bioinformatics tools will be employed to identify biochemical mechanism(s) determining substrate specificity of SETD3 enzyme.

The successful completion of this project will result in the identification of cellular processes requiring SETD3 activity and will shed new light on the physiological importance of protein methylation. Additionally, disclosure of the protein sequence motif recognized by SETD3 enzyme will be helpful in understanding mechanism determining its substrate specificity. Finally, findings of this project will plausibly significantly contribute to the understanding of mechanisms behind pronounced anatomical and physiological disturbances reported in mice devoid of SETD3 gene (http://www.mousephenotype.org/data/genes/MGI:1289184) and might prospectively facilitate the identification of human disorders in which the SETD3 enzyme is involved.