In this project we will attempt to shed some light on the question not raised in any original or review report so far, and stating that COX-1 non-enzymatic modification alone or followed by further ubiquitination are the major factors of increased COX-1 activity, also reflected as elevated COX-1-dependent platelet activation in patients with type 2 diabetes.Our idea assumes that the effect of glycation on the conformation, function and biological fate of platelet COX-1 can, to a great extent, be explained by modification(s) of its crucial activity sites in the molecule.

In the first step of this project in the group of control and diabetic patients we will monitor platelet aggregation (including COX-1-dependent pathway), generation of thromboxane A2 (determined as TxB2 concentration) and direct COX-1 activity to study platelet functioning and to verify whether the diabetic patients developed the 'aspirin resistance', comparing the results of platelet functioning before and after ASA administration and whether the competition between a non-enzymatic glycation and ASA-mediated acetylation of platelet COX-1 occurs in this study group. Next, using LC-MS/MS method we will study whether the COX-1 is glycated or whether the glycation of this enzyme is increased in platelets from diabetic patients, in comparison to healthy volunteers. We will also define the changes of COX-1 acetylation in platelets from healthy and diabetic individuals. We will determine secondary structure of COX-1 isolated from diabetic and control platelets based on the circular dichroism as one of the prefered technique. In the same study groups we will measure concentrations of serum ubiquitin, level of ubiquitinated platelet COX-1, expression of platelet receptor for chemokine 4 and platelet ubiquitination via CXC4 receptor. Therefore we will get to known if there is a correlation between the non-enzymatic modification of COX-1 and ubiquitination of this enzyme in people with
diabetes type 2 and if the higher level of extracellular ubiquitin and/or platelet chemokine receptor 4 correlates with the higher level of
ubiquitinated platelet COX-1 in diabetic patients.

We believe that this will be the first original and well documented research, concerning the possible role of elevated platelet COX-1
ubiquitination in type 2 diabetes. Uncovering the mechanisms of COX-1 modifications caused by glucose is fundamental not only for the understanding of the pathogenesis of some diabetic complications, but mostly for explaining of the phenomenon of 'aspirin resistance' observed in patients with diabetes. A number of factors are considered, which could potentially contribute to the development of an
individual's 'resistance' to this drug. However, there is no direct experimental evidence pointing that vulnerability to ASA originates from a possible competition between a non-enzymatic glycation and ASA-mediated acetylation of platelet COX-1 in the pathogenesis of this phenomenon. We are strongly convinced that this will be the first report revealing the direct correlation between non-enzymatic modifications of COX-1 and its higher ubiquitination, both leading to much lesser sensitivity of the enzyme to the inhibition by ASA in diabetic patients.