Immunosensors, which use antibodies as detecting molecules, are one of the most commonly applied type of biosensors in medical diagnostic, food safety and pollution screening. This type of sensor bases on a highly specific interaction between antibodies and antigen. Detection of an antigen in a samples is possible thanks to antigen capturing by antibodies immobilized on sensor surface. The process of antibodies surface immobilization is a fundamental step of immunosensor interface functionalization protocol. This process requires a special attention due to various possible orientations which antibodies can adapt on the surface: flat-on, side-on, tail-on, head-on. Antibodies surface orientations affect access to binding sites located on Fab fragments and as the result determine antigen binding efficiency. Therefore, understanding and control of orientation of antibodies immobilized on the surface focus a significant scientific efforts. Readily used antibodies immobilization strategy is random immobilization, which can be realized by a simple physical adsorption or covalent binding through amino groups. However, in this case the dominant antibody orientation is determined by a number of factors such as their surface density, reactivity of amine groups as well as protein-protein and protein-surface interactions e.g. electrostatic or affinity interactions. Therefore, the dominant antibodies orientation resulted from the biosensor interface functionalization protocol should be carefully examined. Despite the great scientific interest, the exact determination of the dominant antibodies orientation remains a challenging issue especially on the complex macromolecular surfaces.

To meet this issue the main goal of the proposed project is determination of the dominant vertical orientation of antibodies immobilized on complex biosensing surfaces functionalized with different molecular and macromolecular layers as well as analysis of factors affecting antibodies orientation. Planned research will be realized by application of Time of Flight Secondary Ion Mass Spectrometry and the novel approach of analysis of the antibodies orientation in the function of its surface amount. This strategy of antibodies orientation analysis enables a direct estimation of the dominant vertical antibodies orientation on particular surface. Additionally, the method of White Light Reflectance Spectroscopy will be applied for examination of antibodies adsorption kinetics, determination of the amount of immobilized antibodies and real-time monitoring of antigen binding for biorecognition efficiency evaluation. Surface characterization will be also supported by Atomic Force Microscopy, Spectroscopic Ellipsometry and ELISA method. Dominant antibodies orientation will be examined on complex molecular layers applicable in biosensors such as proteins overlayers, supported lipid layers and polymer brushes. In addition, the impact of factors such as adsorption stage, solution pH and ionic strength on dominant orientation of surface immobilized antibodies will be examined for physical adsorption and covalent binding immobilization strategies.

The expected result of the proposed project is broadening knowledge about dominant orientations adopted by antibody molecules on different surface and deeper understanding of interactions determining antibodies orientation. Molecular and macromolecular layers on solid supports functionalized with antibodies are important systems for a wide range of applications in biosensing and biotechnology. For the effective performance of such applications crucial is a high share of antibody molecules adapting an active orientation enabling antigens binding. Therefore, expected project results will help to improve and optimize protocols of antibodies immobilization for more effective performance of biosensors and other antibody-based biotechnological platforms.