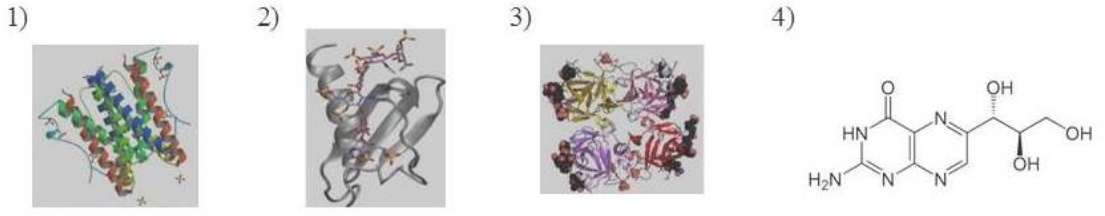
The immune system is composed of a variety of different cell types and proteins. Each element performs a specific task aimed at recognizing and/or reacting against “foreign material”. Disorders of the immune system can result in autoimmune diseases, inflammatory diseases and cancer. The manifestations of immune deficiencies can be a single type of infection or a more global susceptibility to infection There are more than 250 primary immunodeficiency diseases recognized by the World Health Organization. Therefore, development of new method to accumulate knowledge about the organization and function of the human immune system contributes to a better understanding of disorders of the immune system and it opens new avenues for therapeutic regimens. In our work we will use the surface enhanced Raman scattering (SERS)-active nanostructures to gain further insight into the complex interactions four immune markers, neopterin, and selected cytokines (interleukins: IL-6, IL-8, IL-18) with theirs antibodies (Scheme 1) to develop rapid and simple method of monitoring the status of the immune system in body fluids of patients.



Scheme 1. Crystal structures of selected Human interleukin cytokines: IL-6 (1), IL-8 (2), and IL-18 (3), and (4) structural formula of neopterin.

Raman spectroscopy is a technique based on the study of oscillations of molecules and is characterized by high selectivity. In Raman spectroscopy every molecule gives molecular fingerprint specificity and information about the structure of the examined compound. However, the Raman process is relatively weak (approximately only one out of a million photons is scattered in inelastic way). The intensity of the inherently weak Raman bands may increase by six orders of magnitude onto spots created in the gap between two nanoparticles, near sharp edges, tips. Based on that, a new technique, so called surface-enhanced Raman spectroscopy (SERS) has been established. In brief, surface-enhanced Raman scattering is an optical spectroscopy method with high sensitivity and chemical specificity. The phenomenon of SERS is explained by the combination of an electromagnetic mechanism and a chemical mechanism related to charge transfer between a substrate and an adsorbed molecule. The electromagnetic enhancement results from the amplification of light by excitation of surface plasmon resonance (SPR) of the substrate. This huge enhancement of Raman scattering (even single molecules can be observed) ensures that SERS spectroscopy is very effective for ultrasensitive bio-analysis.

Moreover, the concentration of some antigens and/or antibodies in biological fluids is very low in the picomolar or even smaller range. It is possible to reach this limit of detection in SERS, which is not possible in the most of conventionally used nowadays techniques. Additionally, the specific antigen-antibody interactions take place in a very complex physiological conditions with many proteins, peptides, enzymes and other species which finally generate complicated and uncertain results. Therefore, SERS technique with enormous signal intensity and molecular fingerprint specificity with single-molecule sensitivity is a unique and promising powerful tool for studing complex biological samples. However, a standard application of SERS in biological and biomedical analyses is strongly hampered by the lack of substrates which would satisfy the following requirements: high enhancement factor, high stability upon exposure to air, high reproducibility of SERS signals both across a single and between different substrates. Researchers from our group have explored numerous promising substrates that could be used as efficient SERS-substrates. In the proposed project we will use silver-gold bimetallic surface prepared by electrochemical deposition of gold over an electrochemically roughened silver surface [Patent application: P-406026 (2013)]. This particular SERS structures are competitive with commercial substrates (Klarite) and may contribute to broadening the medical diagnostics, especially immunological diseases.

SERS detection of biomolecules has been accomplished in both intrinsic and extrinsic formats. In intrinsic SERS biosensing, the molecular signature for the analyte of interest is acquired directly from SERS nanostructures. In the proposed project, this strategy will be applied for direct detection of neopterin, one of most important immuno-marker. Concentration of neopterin in body fluids indicates the state of activation of the cellular immune system during subsequent stages of various diseases, such as autoimmune diseases and viral infections (hepatitis A, B, and C, cytomegalo, measles, rubella, influenza) and bacterial infections, cardiovascular disease , insulin resistance, allograft rejection, and some tumors. In our project, for the first time, we will elaborate novel recognition units based on SERS spectroscopy as fast and sensitive method to study neopterin level in relation to selected bacterial infections like bacterial pneumonia.

For the three cytokines (IL-6, IL-8, IL-18), their qualitative and quantitative analysis will be carried out by indirect analysis using the so-called SERS "Raman reporters," i.e. molecules, mainly dyes, which give a very strong Raman signals. The spectral image of the "Raman reporter" will indicate the presence of the analyte in measured sample. Cytokines are one of the major diagnostic markers in various disorders of the immune system. These small proteins serve as hormones for the immune system and play important roles in cell signaling. Elevated levels of these all three cytokines (interleukin IL-6, IL-8, IL-18) are observed in the serum of patients with breast cancer and can be correlated with the clinical stage of this disease.

Besides presented above practical applications the proposed project offers both the basic understanding of processes linked with antigen–antibody interactions as well as understanding the complex interactions between these molecules and plasmonic materials (SERS nanostructures). The efficiency of formation of antigen-antibody complex is affected by the following factors: pH, temperature, time, ionic strength, and molecular orientation of the immobilized antibody on the SERS substrate. The impact of the mentioned above parameters is going to be extensively studied using the SERS spectroscopy. In the framework of this project we will also develop metal nanostructures and optimize their sensitivity, stability and reproducibility of recorded signals. In addition, we will develop (1) a method of the modifications of SERS-nanostructures with thiols monolayers, (2) the method of

immobilization of suitable monoclonal antibodies onto linkage layers of thiols, (3) method of gold nanoparticles synthesis and their subsequent modification with selected Raman reporters molecules and/or monoclonal antibodies (4) incorporation of SERS-nanostructures into designed microfluidic devices to perform simultaneous, qualitative and quantitative analysis of neopterin and cytokines in tested samples.

Several measurement techniques available in our Institute such as atomic force microscopy (AFM), scanning electron microscope (SEM), surface plasmon resonance (ang. SPR) or dynamic light scattering (DLS) will be used for implementation of research plans. To analyze the complex spectral data (from multiplex measurements) the numerical chemometric methods based on e.g. principal component analysis (PCA) will be applied.

To summarize, the proposed project is linked to challenging and intensively developed, both in Europe and other countries, field of science and technology concerning novel "platform" for chemical and biological analysis with unprecedented routine levels of sensitivity, specificity and reproducibility. Development of novel technologies, including preparation of nanomaterials and functional materials in particular for applications in the health care and clinical diagnostics is considered by European Commission as one of the strategic direction. Our project is intimately linked to the fields mentioned above, as we are undertaking development of novel materials and methods for selective recognition of immune disease markers. Our work will offer both, basic understanding of processes linked with antigen–antibody interactions as well as understanding of the complex interactions between these molecules and plasmonic materials, and practical applications of the developed immune sensing devise in the field of analytical chemistry. These applications may be further developed into the practical prototypes. Moreover, due to possibility of use the low-cost and portable Raman spectrometer, which can be easily adapted in clinical environments, Raman spectroscopy demonstrate a great potential in immunoassay applications, also from the economical point of view.