The scientific aim of this project is to use the phage display technique to isolate bacteriophage/peptide that specifically recognize C-reactive protein (CRP) - a marker of inflammatory processes in human body and use them as artificial antibodies for the differentiation between viral and bacterial infections.

C-reactive protein belongs to a class of acute-phase proteins. Its increased concentration in blood indicates the state of ignition in the human body. Its testing is essential for monitoring and diagnosing various diseases e.g. cardiovascular and autoimmune. Moreover, its testing is essential also for the differentiation between viral and bacterial infection which is important especially with the advent of antimicrobial resistance. Commercially available CRP test is expansive, which limits its accessibility. The approach of using bacteriophage particles and phage display method for the identification of **new, cheaper CRP receptors proposed in this project** could be the solution to improving the availability of the CRP testing. These new receptors, in turn, can be used for constructing new, low-cost and sensitive sensing platforms. In general, the function of most of the currently available diagnostic is to measure the concentrations of characteristic markers. Usually, as a detection layer, that determines selectivity and sensitivity of such tests, antibodies are used - and so it is in CRP test. Production process of these antibodies is expensive and difficult as it requires conducting immunizations of animals. There are also issues with their sensitivity and resistance to external factors. It is therefore desirable to find new biomolecules that can be used to fabricate better sensing layers**. These problems can be circumvented by employing artificial antibodies - organic compounds (e.g. peptides)** responsible for binding specific particles/markers but having less complex structure than classical antibodies. These **artificial antibodies** like peptides can be produced quickly and cheaply, and are in general more stable and robust to external factors than the antibodies. Their sensitivity towards studied particles is comparable with antibodies' sensitivity or is even better. Over the last decade, bacteriophages (in short "phages") particles have been successfully used as **artificial antibodies.** Phages are viruses of the bacteria (harmless to humans). They are ubiquitous – it is estimated, that there are between 10^{30} and 10^{32} particles of them on the Earth. In biology they serve as model particles, and it was phages which led to the proof, that DNA contains genetic information. Currently they have found applications in environmental analysis as an indicator of water contamination or in medicine as an alternative for antibiotics (phage therapy). Phages can also be used for particle/marker detection - using the phage display method. In this project the phage-display method will be utilized to identify phages/short peptides binding to CRP. The phage display method has been developed in molecular biology and used for instance for the development of proteins exhibiting desired binding affinity towards particular molecules/compounds. Later it was adapted to the development of new materials and structures. The method is often used in conjunction with so called phage libraries. Phage libraries are ready to use solutions containing a mixture of a large number (billions) of diverse variants of bacteriophages. The phage display method used with phage library operates by selecting from a given library, clones (types of phage) which bind target marker, the affinity to which the researcher wants to obtain – so called antigen. The selected phages with its respective peptide, which exhibits the highest binding efficiency and selectivity towards CRP will be characterized using biological and physicochemical methods. In that aim, new receptors will be attached to a suitably modified substrate/support. When a substrate modified with the new CRP binding receptor is immersed in a sample containing CRP molecules, some of these will be caught by these artificial antibodies. That result in a change of the measured electrical or optical signal of the substrate modified with a layer of artificial antibodies enabling the measurement of CRP in the test samples. From the literature it is known that the phage display technique makes it possible to identify phages/peptides binding diseases markers (*Biotech&Bioeng.*, 105 (2010) 678, *J. Biotech.*, 187 (2014) 43). Later research shows that such peptides can then be sequenced and synthesized outside of a phage, and used for preparation of detection layers enabling sensitive and selective detection of these markers (*Anal. Chem.*, 82 (2010) 8235, *Anal. Sci.*, 31 (2015) 699). These observations have contributed to the proposed research subject.

Analysis of the described issues will allow to determine whether peptide/phage have higher affinity towards CRP than antibodies **and if the support modified with such phages/peptides are sufficiently sensitive and selective to be used for fast and cheap differentiation between viral and bacterial infections**. It is also because of this issue that the principal investigator undertook this research subject. The proposed research will extend basic knowledge in the area of development of new recognition elements for new sensing platforms, and molecular biology of phages and in the phage display technique. New CRP binding peptides identified during this project could be an interesting alternative for antibodies and become a kingpin for constructing new, low-cost and sensitive sensing platforms. The knowledge about the generation of the new receptors which bind C-reactive protein and their characterization for molecular recognition will also be collected.