1. SCIENTIFIC GOAL OF THE PROJECT

Nitric oxide (NO) is one of the major transmitters involved in maintaining of the proper vascular homeostasis in the circulatory system through contribution in lowering blood pressure and vasodilating smooth muscle cells. Bioactivity of NO is controlled, among the others, by heme proteins which participate in both – promoting and suppression of NO vasodilating property. Significance of heme proteins depend on the oxidation state of porphyrin-bound iron ion – **ferrous heme proteins** (with Fe^{2+} iron ion) **scavenge and attenuate NO** while **ferric heme proteins** (with Fe^{3+} iron ion) **interact reversibly with NO allowing its diffusion and permitting bioactivity**. However, precise role of ferric heme proteins remain elusive due to the lack of methodology allowing for simultaneous detection, differentiation and spatial distribution characterization of ferrous and ferric heme proteins. The aim of this project is to design the **unique imaging methodology based on resonance Raman (rR) spectroscopy** allowing **examination of unexplored aspects of NO bioactivity regulation by ferric heme proteins** and **better understanding of mechanisms regulating NO signaling in erythrocyte and vessel wall**.

2. PROJECT METHODOLOGY

The methodology of the project will be specially designed based on rR imaging with 405 nm excitation wavelength (resonance enhancement of heme proteins signal) supported with Raman imaging with 532 nm excitation (signal from lipids and other proteins present in sample) and polarization measurements (change of the incident light plane toward additional structural information). Such unique combination allow conducting **measurements in completely label-free manner without requirement of any prior sample preparation**, which results **provide information about presence of heme proteins, their differentiation on ferrous and ferric species together with their precise spatial distribution within measured sample**. The designed methodology will be correlated with the well-established histological and immunohistochemistry methods. Impact of the ferric heme proteins on the NO signaling will be additionally tracked with techniques such as: UV-Vis absorption spectroscopy, EPR spectroscopy, ELISA and chemiluminescence.

3. JUSTIFICIATION OF THE PROJECT' SCIENTIFIC SUBJECT

Discovery that unknown in 80s endothelium-derived relaxing factor (EDRF) is nitric oxide was important breakthrough in cardiovascular medicine. The main clue in this finding was hemoglobin – heme protein which in its ferrous state is not only able to reversible bound oxygen, but also scavenges and inactive NO bioactivity. However, as it later occurred, hemoglobin in ferric state is also able to interact with NO leading to formation of unstable adduct, which allows NO diffusion and permits its vasodilatory function. Therefore, it was concluded, that ferric heme proteins may play a role in physiology acting as NO transporters (e.g., as adducts) or as intermediates in formation of more stable complexes (e.g., SNO-Hb). Recent years revealed other heme proteins, such as α-globin and cytoglobin, which maintaining in theirs ferric states remain crucial for NO bioactivity. However, despite extensive studies in the field **it remains elusive, whether maintaining of NO bioactivity in erythrocyte and vessel wall relies only on lack of ferrous heme proteins** (which scavenge and shut off NO) or **presence of ferric heme proteins *per se* is the key** (which permits and supports NO).

4. EXPECTED IMPACT OF THE PROJECT

Realization of the project will allow to **design the unique rR imaging methodology for detection, differentiation and spatial distribution characterization of ferrous and ferric heme proteins**, what will shed the new light on **importance of ferric heme proteins in context of NO bioactivity** in circulatory system as well as **better understanding of this extraordinary alliance between NO signaling and erythrocyte and vessel wall**.