The aim of the project is to study an influence of endothelial gaseous mediators, **nitric oxide** (NO), **carbon monoxide** (CO) and **hydrogen sulfide** (H_2S) on porphyrins in various human- and murine derived biological samples. Especially, the interaction **between the iron ion in hemoglobin of red blood cells and NO, CO and H_2S** will be reevaluated, as well as with **detection of the formed Hb-X adducts,** which could have a potential importance in these gasotransmitters bioavailability. The principal assumption of the project is to carry out experiments ***ex vivo*** (whole human blood and isolated human red blood cells, murine isolated Kupffer cells) and ***in vitro*** (murine macrophage cell line) on biological samples instead on standard compounds. Due to the used methods (resonance Raman spectroscopy and spectrophotometry UV-Vis), it will be possible to deeply inquire into the changes both on molecular level and on quite bigger, macroscopic scale of studied systems. All the measurements will be done in pH and temperature controlled environment to the best reproduction of physiological conditions.

Resonance Raman Spectroscopy (RRS) is a technique of vibrational spectroscopy, which is a convenient tool to study biological samples, as the measurements can be done in water environment without any threat to measured sample. Moreover, due to the unique properties of RRS to enhance Raman signal of different part of molecule in dependence on used excitation wavelength, there is possible more accurate analysis of envisaged in the project interactions.

UV-Vis spectrophotometry is from the other hand very fast and easy technique. For the absorption spectra of hemoporphiryns in the range of 300 – 700 nm there are few characteristic bands (Soret band in the range 400 – 440 nm and Q bands in 500 – 600 nm) which localization, exact number and even intensity ratios allows to detailed analysis of molecular level changes, i.e. oxidation state of the iron ion or presence of ligand in axial positions of the iron ion in porphyrins.

All above mentioned issues were studied mostly with the usage of standard compounds. The techniques which were proposed in the project will allow to carry out experiments on biologic systems without danger of destroying the samples and involving changes coming from methodology itself. Due to the special chamber for physiological RRS measurements, we will have full control over environmental conditions such as temperature or oxygen saturation level (hypoxia/normoxia) and actually great tool to making experiments *in vtiro* and *ex vivo*. Results combined from both spectroscopic techniques will allow than to **molecular analysis of hemoglobin–NO, –CO and –SH_2 interactions in biological samples *in vitro* and *ex vivo* and for detection and differentiation formed Hb-X adducts.**