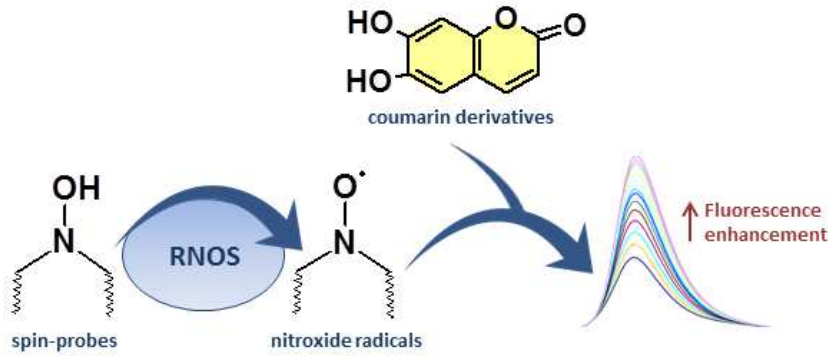
The main goal of the research project is a designing of an innovative method for the measurement of the total amount of different reactive nitrogen and oxygen species (RNOS), being precursors of the oxidative stress. The method will be based on the identification and then quantitative determination, namely with the use of high performance liquid chromatography, fluorescence spectroscopy, and dihydroxy-substituted coumarin derivatives as fluorescent sensors, of the nitroxide radicals – formed by reactions of different „spin-traps" (hydroxylamines, nitrone and nitroso compounds) with the RNOS studied. The insertion to a buffer (model) solution containing a palette of reactive nitrogen and oxygen species of appropriate „spin-traps" and the subsequent quantitative measurement of the nitroxide radicals formed will constitute a sensitive, accurate, relatively cheap and fast indirect method for the measurement of oxidative stress status in the probe studied. Additionally, the same experiments will be performed in biological material – extracts of the breast cancer cells cultured under different conditions, among others under oxidative stress caused by ionizing radiation. The scheme below constitutes a graphical description of the studies being the heart of the proposal.



Firstly, the reactions between a group of "spin-traps" (hydroxylamines, nitrone and nitroso compounds) and different reactive nitrogen and oxygen species will be studied. The products of the reactions will be identified and possible mechanisms proposed. The structures of the nitroxide radicals formed will be confirmed by the use of their commercially available standards. The selectivity of hydroxylamines, nitrone and nitroso compounds towards particular reactive nitrogen and oxygen species will be evaluated. Secondly, the type of interactions between nitroxide radicals (formed as products of the reactions of the spin probes with RNOS) and selected fluorophores (dihydroxy-substituted coumarin derivatives) will be defined. In case of strictly physical interactions the mechanism of fluorescence quenching will be defined (qualitatively and quantitatively) and quenching rate constants will be determined. In case of chemical reactions between the coumarin derivatives and the nitroxide radicals, their kinetics and mechanisms will be established. The structures of the products of the reactions will be determined. Experimental concentrations of the nitroxide radicals formed will be evaluated from the previously prepared calibrations curves. Finally, an insertion of selected "spin-traps" to a solution containing fixed amounts of different reactive nitrogen and oxygen species and then subsequent quantitative determination of the nitroxide radicals formed (on the basis of their interaction with an appropriate coumarin derivative) will make it possible to establish precisely the total content of these RNOS. The concentrations of spin-probes used in the experiment will be established individually to guarantee the trapping of all amounts of each RNOS under basal conditions (the absence of reactive nitrogen and oxygen species in the probe after their reaction with appropriate spin-probes will be confirmed by the use their commercially available fluorescent sensors). Additionally, the interactions between coumarin derivatives studied and compounds being potential interferents under biological conditions will be defined. To evaluate the usefulness of the presented method for the measurement of oxidative stress status, the studies will be performed in biological material. Appropriate extracts for these measurements will be obtained through the lysis of human breast cancer cells (MCF-7 line) cultured in three variants: (i) under normal concentrations of oxygen, (ii) under normal concentrations of oxygen and exposed to ionizing radiation, what induces oxidative stress, and (iii) under hypoxic conditions.

The realization of the project will undoubtedly start studies on searching for new biosensors which will bind with RNOS and neutralize their negative action more effectively than selected "spin-probes". Results obtained with the use of the proposed method may be applied in biological studies in order to identify in cells places where the level of reactive nitrogen and oxygen species is alarming, as well as for determination of terminal concentration of RNOS, when side effects of oxidative stress are observed. As a consequence they may have an influence on the development of methods for diagnosis and treatment of different affections, such as: diseases of vascular system, neurologic disorders, inflammable articulations diseases or lungs diseases. Proposed in the project method for determination of reactive nitrogen and oxygen species may be applied in biotechnology, medical diagnosis and pharmaceutical industry.