Reactive oxygen species include among others: hydrogen peroxide (H_2O_2), hydroxyl radical ($HO^{\bullet}$), alkyloxyl radical ($RO^{\bullet}$), alkylperoxyl radical ($ROO^{\bullet}$), superoxide anion ($O_2^{\bullet-}$), hypochlorite anion (OCl^-) and singlet oxygen ($^1O_2$), whereas among reactive nitrogen species nitric oxide (NO), nitrogen dioxide (NO_2) and peroxynitrite anion ($ONOO^-$) can be mentioned. Reactive nitrogen and oxygen species (RNOS), being precursors of the cellular stress, are incessantly produced as a result of a metabolism. Although RNOS participate in many physiological processes and play a very important role in cells communication, in excess they contribute to the pathogenesis of many diseases. A negative influence of an overproduction of reactive nitrogen and oxygen species on a physiology proves that search for their potential biosensors, enabling their quantitative determination and disposal, is of great importance.

The studies were performed with the use of the following analytical techniques: UV-Vis spectrophotometry, high-performed liquid chromatography coupled with mass spectrometry and fluorescence spectroscopy, both steady-state and time-resolved.

One of the aspects of my studies was a comprehensive characteristics of the interactions between the chosen RNOS and 1,3-diphenylisobenzofuran (DPBF), a fluorescent probe which was believed for almost 20 years to react highly specifically towards some reactive oxygen species such as $HO^{\bullet}$, $RO^{\bullet}$, $ROO^{\bullet}$ and $^1O_2$. According to literature data the product of the reaction between DPBF and these reactive oxygen species is 1,2-dibenzoylbenzene. I decided to check if DPBF gives the same product under the action of other reactive oxygen and nitrogen species, and as a result if it can play a role of a potential molecular biosensor of a total amount of RNOS. For this purpose I have examined the type of interactions between 1,3-diphenylisobeznofuran and five (never studied before) commonly present under biological conditions reactive nitrogen and oxygen species (NO_2, $ONOO^-$, $O_2^{\bullet-}$, OCl^-, H_2O_2) as well as anions NO_2^- and NO_3^-. I have proved that in case of the reaction of DPBF with NO_2, $ONOO^-$, $O_2^{\bullet-}$, OCl^-, NO_2^- and NO_3^- the product is 1,2-dibenzoylbenzene, as well. I have revealed that in case of the interactions between DPBF and H_2O_2 the product of the reaction is

biphenyl-2,2’-dicarbaldehyde. Additionally, I have studied the kinetics of that reaction and determined limits of detection and quantification of hydrogen peroxide with the use of DPBF. From the results obtained, it should be stressed that 1,3-diphenylisobenzofuran is highly specific towards hydrogen peroxide, what enables its potential use in biological studies to determine the concentration of H_2O_2.

Another aspect of my studies was the determination of the interactions between a series of 16 fluorescent probes and a stable nitroxide radical 4-hydroxy-TEMPO, the most widely studied in terms of interactions with RNOS derivative of 2,2,6,6-tetramethylpiperidine. The main goal of these studies was to find a compound which reacts with 4-hydroxy-TEMPO selectively or specifically and as a result enables the quantitative determination of the radical. The interactions between a vast majority of the studied compounds (polycyclic aromatic hydrocarbons, fluoroquinolone antibiotics and the majority of coumarins) and 4-hydroxy-TEMPO have been strictly physical. For these compounds I have determined the mechanism of fluorescence quenching (dynamic quenching), Stern-Volmer quenching constants and bimolecular quenching constants. Only in the case of 6,7-dihydroxycoumarin and few other derivatives of coumarin with two hydroxyl groups I have registered clear changes in absorption and emission spectra of the fluorophores under the action of 4-hydroxy-TEMPO (namely increase in fluorescence intensity and slight shift of the emission maximum towards longer wavelengths), what proves that chemical reaction has occurred. I have proved that under the action of 4-hydroxy-TEMPO coumarin dimerizes – the product was identified with the use of high-performed liquid chromatography coupled with mass spectrometry. Furthermore, I have determined the range of 4-hydroxy-TEMPO concentrations, for which the linear increase in fluorescence intensity was observed and the limit of 4-hydroxy-TEMPO detection. Additionally, I have confirmed the selectivity of 6,7-dihydroxycoumarin towards 4-hydroxy-TEMPO. The completion of these studies enables quantitative determination of 4-hydroxy-TEMPO

and constitutes a first stage of the direct method for quantitative determination of the total amount of RNOS in biological probes.