# Preface

The present volume is the second in the continuation of the new collection of oxidative stress (OS) protocols that focus on novel techniques for detecting ROS/RNS, unique AOX technology and applications, gene expression and biostatistics for evaluating OS-derived experimental data. It presents 30 additional chapters to Volume I. These current methods expand on those in *Advanced Protocols I*, giving researchers the opportunity to have 61 total chapters at their disposal. The number of ROS/RNS and AOX chapters in Volume II is now roughly equal, indicating a trend toward therapy.

Once again, there is broad international participation. This volume includes animal models and numerous studies focusing on mitochondria during hypoxic conditions using advanced methods for $pO_2$, peroxynitrate, reactive S-nitrosothiols, lipid peroxides, COX and the mitochondrial membrane potential. Cell permeable fluorescent and chemiluminescent probes directly measure the global redox state using flow cytometry, and mutiphoton microscopy is described for metabolic monitoring which requires no sample preparation. Erythrocyte fragility can also be used to analyze the effect of OS relative to change in membrane structure. A PubMed search shows that there were over 40 publications relating to OS and mitochondria in January 2009 alone. It is predicted that studies on mitochondrial OS will increase and represent a major portion of future issues of *Advanced Protocols.*

The format is the same as in Volume I, and chapters in Part I present point-of-care in vivo testing in many diverse clinical settings for use in diagnosis, prognosis, and management. It is envisioned that a "gold standard" using a combination of ROS and AOX biomarkers in body fluids can be identified and confirmed. This will be valuable in giving clinicians a global assessment of redox status in their patients. In fact, many bench tests have already been converted into commercial kits for this purpose which are reproducible, fast, user-friendly, and cost effective. These assays can be applied for real-time monitoring.

Chapters in Part II suggest the use of various ROS and AOX biomarkers in developing an index for summarizing OS status. Comparisons between biomarkers including ORAC, TRAP, FRAP, ABTS, DPPH, TOSC, TEAC, PMRS, and oxHLIA are described, and combinations of several biomarkers are recommended for optimal results. Other new methods are presented using cupric ion assays for hydroxyl radical detection, laser-induced breakdown spectroscopy for trace metals, oxidative hemolysis, AOX hybrids of lipoic acid that increase potency, and pentaerithrityl tetranitrate (PETN) therapy to decrease the OS burden. Bacterial biotransformation can be used as an alternative method to pharmacological synthesis for preparation of estrogenic AOX compounds. Electrochemical detection with a new boron-doped diamond electrode is described for quantifying reduced and oxidized glutathione and for thioesters. A plasma membrane redox system is presented for recycling ascorbate radicals. Turmeric is shown to be effective in reducing lipid peroxides and also has immunomodulating properties.

Chapters in Part III illustrate purification of AOX gene vectors for delivery of large amounts of AOX enzyme DNA, thus increasing new in vivo synthesis in the treatment of metabolic disease. The activation of caveolin-1 promoter by ROS is detected using EMSA

and chromatic immunoprecipitation methodology, where the latter technique can actually pinpoint transcription factors.

The last chapter gives a statistical approach using meta-analysis that polls data when there is conflicting or inconclusive results, thus providing more accurate analysis of therapeutic intervention. Taken together with methods published in previous books, i.e., volumes 108, 186, 196, and 477, there are now a total of 169 technologies available to readers on OS in this series. These volumes provide a valuable office and laboratory resource for OS methodology. To emphasize the importance of OS technology, PubMed lists 643 articles linking free radicals with advanced technology and 801 linking AOXs with advanced technology.

Chapters in the *Advanced Protocols* series can be used in graduate education, and the preparation of them is an excellent opportunity for junior researchers to gain experience in writing in a clear and organized manner.

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