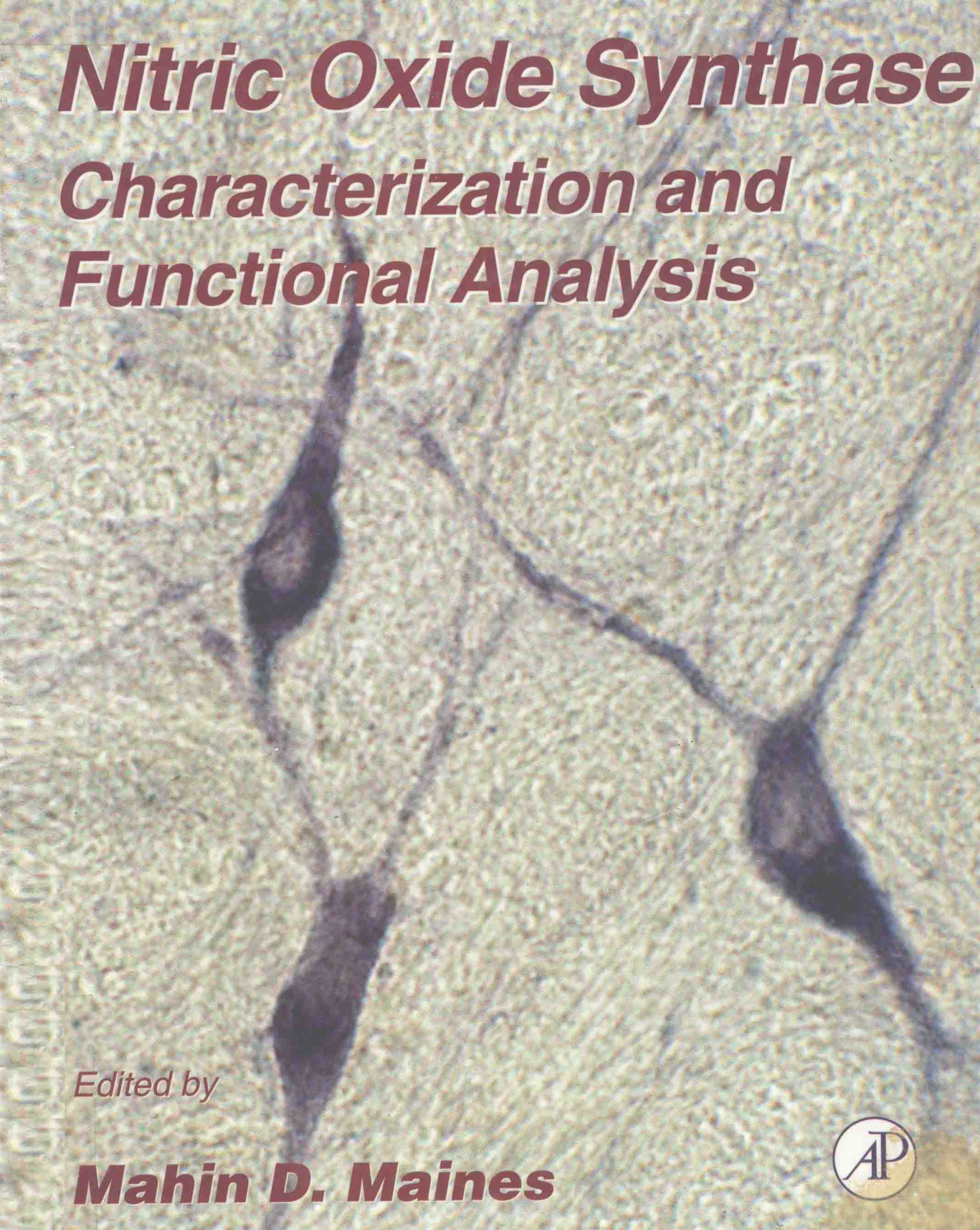
Nitric Oxide Synthase

Characterization and Functional Analysis



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Preface

Because of the immense interest in the biological function of nitric oxide (NO), this volume is devoted to this molecule and related components. This simple molecule, which acts in a fairly specific manner, is now recognized as an integral part of various homeostatic mechanisms, including the central and peripheral nervous system and cardiovascular system, as well as host–defense interactions, where it can directly alter vital functions. The impact of the biological actions of NO is further augmented by its activity that affects gene expression. The unwavering interest in NO relates to the seemingly unending and astonishing diversity of cellular and physiological functions that are attributed to it. The role of NO in the nervous system is of particular interest because, unlike neurotransmitters such as GABA, dopamine, and glutamate, which have highly restricted regional distribution, the ability to produce NO is found in many cells in the brain. And, because NO can diffuse across membranes, its range of activity far extends its local effects. As the significance of NO activity in the brain becomes more apparent, the methods that allow investigators to identify its role in cellular and physiological functions become invaluable.

In assembling this volume, I have attempted, in cooperation with a distinguished group of contributors, to produce a useful tool for neuroscientists and other investigators in the field of NO and those entering it. I am grateful to the contributors for their cooperation and thankful to the staff of Academic Press, particularly Shirley Light, for their support and assistance.

MAHIN D. MAINES

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Section I

Enzyme Activity Measurements, Nitric Oxide Detection, and Tissue Localization

[1] Electron Paramagnetic Resonance Detection of Nitric Oxide in Biological Samples

Lori G. Rochelle, Harriet Kruszyna, and Roger P. Smith

Introduction

Nitric oxide (NO), a small inorganic gas molecule, can freely diffuse in and out of cells and tissues. The physicochemical properties of NO including high diffusibility and reactivity result in a short biological half-life and make detection and quantification of NO difficult under physiological conditions. Electron paramagnetic resonance (EPR) is a method that can be used to detect molecules with unpaired electrons (paramagnetic molecules) such as NO. Free NO is not detected by EPR because of its rapid relaxation time, but it is possible with this technique to take advantage of spontaneous endogenous reactions for binding and stabilizing NO in extracellular or intracellular locations. Nitric oxide interacts with the constituent sulfhydryls and transition metals of a variety of endogenous molecules. Electron paramagnetic resonance has been a particularly useful technique for detecting NO bound to iron proteins, which constitute some of the predominant cellular NO complexes. Binding to iron-containing molecules such as hemoglobin permits not only detection, but also stabilization, characterization, and quantification by EPR. Nitric oxide interacts with endogenous molecules integral to the exterior or interior of the cell or with exogenous materials that may or may not be taken up by the cells.

Electron paramagnetic resonance is an especially attractive method for detecting NO in biological samples because under some conditions, the sample can be directly introduced without further preparation into the EPR tube for immediate analysis or snap frozen in liquid nitrogen for future analysis. Most other components of cells and tissues are invisible to this technique, which provides the necessary specificity for the NO-bound iron complexes discussed in this chapter.

Electron Paramagnetic Resonance Spectroscopy

Electron paramagnetic resonance spectra are produced by energy absorbances analogous to UV/Vis or IR spectroscopy. In this technique, the energy source emits microwave frequencies that are absorbed by unpaired

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