# Preface

In this book, the authors present current research in the study of the structure, biology and clinical significance of calpain, enzymes and enzyme activity. Topics discussed include monitoring the activation of calpain I for sensitive assessment of pathogenesis and neuroprotectant action; the myeloperoxidase enzyme as a diagnostic biomarker and target for new drug developments; the biological effects of calpain inhibitors on human phagocyte functions; ALDH activity of Artemia as a tool for the investigation of the toxicity of antifouling paints; and calpain as an indicator of organo-phosphorous-induced delayed neuropathy.

Chapter I – Myeloperoxidase (MPO, EC 1.11.2.2) is an iron-containing peroxidase enzyme released primarily by neutrophils and, to a lesser extent, by monocytes and some tissue macrophages. The MPO unique ability to catalyze the hydrogen peroxide ($H_2O_2$) dependent oxidation of chloride ($Cl^-$) to hypochlorite ($ClO^-$) – the most reactive oxidant produced by neutrophils – accounts for its primary function as a bactericidal enzyme linked to the innate host defense. Nevertheless, its relevance in the pathophysiology of numerous diseases has become evident only in recent years. For example, circulating levels of MPO have been shown to predict risks for major adverse cardiac events, whereas the levels of MPO-derived oxidants serve as specific biomarkers for disease progression. In addition to cardiovascular diseases, many clinical and experimental studies have implicated MPO in cancer, neurodegenerative disorders or lung diseases, among others. In this chapter, MPO structure and complex catalytic cycle, as well as the intrinsic role in health and diseases are reviewed. A concise overview on the future perspectives of this enzyme as a diagnostic biomarker and target for new drug developments is also provided.

Chapter II – Calpain is a calcium-sensitive protease whose over-activation has been linked to a variety of degenerative conditions. This chapter will focus on calpain's involvement in neurodegeneration, however its pathogenic role is not restricted to the nervous system. It has been implicated in cardiac pathology, kidney hypoxia, respiratory disorders, myopathy, bone erosion, and eye diseases. Of the many substrates that calpain cleaves in brain and peripheral tissues, the cytoskeletal protein αII-spectrin has been identified as being useful to measure pathogenic calpain activation during cellular toxicity and certain diseases. Calpain-mediated spectrin breakdown has been used extensively to monitor cytoskeletal damage, one of the sensitive precursors of neuronal pathology found in animal models of excitotoxic insults and human brain injury. Cleavage of spectrin by calpain generates a distinct set of large breakdown products (BDPs) that are unusually stable. Detection and measurements of BDPs have allowed calpain-mediated proteolysis to be established as an early event contributing to the neurodegeneration associated with ischemia, hypoxia, genetic defects, vasospasm, traumatic brain injury, and seizures, and it may be involved in brain aging and Alzheimer-type pathogenesis. Being sensitive markers of excitotoxicity and other types of cellular deterioration, the spectrin fragments make up an important assay of pathology using specific labeling by antibodies against the cleavage site recognized by calpain. The ability to detect the earliest signs of pathology in experimentally-induced models of disease states allows for the careful screening of potential therapeutic agents. Especially in brain tissue targeted by acute, definable neuronal insults, neuroprotectants can be assessed for their ability to reduce calpain-mediated cytoskeletal damage and associated damage to central synapses. Excitotoxic calpain activation is commonly associated with reductions in synaptic markers, particularly among pyramidal neurons of the hippocampus. In fact, calpain-mediated spectrin breakdown levels have been found to correspond with synaptic deterioration as well as with subsequent cell death in the hippocampal CA1 subfield. The excitotoxic hippocampal slice model is often utilized for neuroprotectant characterization and screening, in which slice cultures are briefly exposed to an excitotoxin, and the neuronal stimulation subsequently quenched with glutamate receptor antagonists. Potential neuroprotectants, applied to the slices before or after the insult, are tested for effects on cytoskeletal proteolysis and synaptic decline evident 2-48 hours later. Monitoring spectrin breakdown and synaptic markers in *in vitro* and *in vivo* disease models is a valuable first step in identifying lead compounds for therapeutic indications. They are also sensitive measures for preclinical toxicology testing.

Chapter III – The intracellular proteases have several functions in regulating various cellular systems. Among them, proteases that are activated by calcium (i.e. calpain), which is a second messenger in cell regulation. Proteases duties consist mainly in the degradation of cytoskeletal proteins, modification of steroid receptors and activation of protein kinases. It has been established that there are at least two types of calpain. One type requires calcium concentrations in the order of mM for their activity, while the other type requires calcium concentrations in the order of μM. Organophosphorus (OPs) compounds, which include pesticides, industrial chemicals and some medicines, are the principal class of insecticides and chemical warfare nerve agents. They have become the most widely used insecticides in the world since the 1970s. Clinically, the symptoms of OP poisoning are divided into four categories: acute cholinergic syndrome, intermediate syndrome, chronic organophosphorus-induced neuropsychiatric disorders (COPIND) and organophosphorus-induced delayed neuropathy (OPIDN). OPIDN has been observed in more than 70,000 human cases in the last 75 years and it has been known since the 1930s when tuberculosis patients were treated with phosphor-creosote, an uncharacterised mixture of esters derived from phosphoric acid and coal-tar phenols. In 1930, thousands of paralysis cases occurred in the United States due to the consumption of Jamaica ginger extract contaminated with tri-ortho-cresyl phosphate (TOCP). Later, similar epidemics were reported in Morocco, Netherlands, Yugoslavia, France, South Africa, Sri Lanka and India. OPIDN is a neurodegenerative disorder characterised by ataxia progressing to paralysis with a concomitant central and peripheral distal axonopathy, and this neuropathology occurs similar to Wallerian-type degeneration. Initially, neuropathy target esterase (NTE) phosphorylation is involved and then an imbalance in free calcium homeostasis occurs, which leads to the activation of calpain. This sequence of events promotes protein digestion in the terminal axon. Calpain activation has been used to identify Wallerian-type axonal degeneration and this biomarker has been related with the appearance of lesions in long tracts of central and peripheral nervous system. These lesions have caused signs and symptoms that include tingling of the hands and feet. This tingling is followed by sensory loss, progressive muscle weakness and flaccidity of the distal skeletal muscles of the lower and upper extremities and ataxia, which appear about 8-14 days after poisoning. Therefore, calpain evaluation became an excellent tool to indicate axonopathies that affects people around the word.

Chapter IV – The intracellular proteases have several functions in regulating various cellular systems. Among them, proteases that are activated by calcium (i.e. calpain), which is a second messenger in cell regulation. Proteases duties consist mainly in the degradation of cytoskeletal proteins, modification of steroid receptors and activation of protein kinases. It has been established that there are at least two types of calpain. One type requires calcium concentrations in the order of mM for their activity, while the other type requires calcium concentrations in the order of μM. Organophosphorus (OPs) compounds, which include pesticides, industrial chemicals and some medicines, are the principal class of insecticides and chemical warfare nerve agents. They have become the most widely used insecticides in the world since the 1970s. Clinically, the symptoms of OP poisoning are divided into four categories: acute cholinergic syndrome, intermediate syndrome, chronic organophosphorus-induced neuropsychiatric disorders (COPIND) and organophosphorus-induced delayed neuropathy (OPIDN). OPIDN has been observed in more than 70,000 human cases in the last 75 years and it has been known since the 1930s when tuberculosis patients were treated with phosphor-creosote, an uncharacterised mixture of esters derived from phosphoric acid and coal-tar phenols. In 1930, thousands of paralysis cases occurred in the United States due to the consumption of Jamaica ginger extract contaminated with tri-ortho-cresyl phosphate (TOCP). Later, similar epidemics were reported in Morocco, Netherlands, Yugoslavia, France, South Africa, Sri Lanka and India. OPIDN is a neurodegenerative disorder character-ised by ataxia progressing to paralysis with a concomitant central and peripheral distal axonopathy, and this neuropathology occurs similar to Wallerian-type degeneration. Initially, neuropathy target esterase (NTE) phosphorylation is involved and then an imbalance in free calcium homeo-stasis occurs, which leads to the activation of calpain. This sequence of events promotes protein digestion in the terminal axon. Calpain activation has been used to identify Wallerian-type axonal degeneration and this biomarker has been related with the appearance of lesions in long tracts of central and peripheral nervous system. These lesions have caused signs and symptoms that include tingling of the hands and feet. This tingling is followed by sensory loss, progressive muscle weakness and flaccidity of the distal skeletal muscles of the lower and upper extremities and ataxia, which appear about 8-14 days after poisoning. Therefore, calpain evaluation became an excellent tool to indicate axonopathies that affects people around the word.

Chapter V – Calpain has been implicated as a regulator of actin cytoskeleton, cell migration, apoptosis, and inflammation, and calpain is a potential therapeutic target for various disorders, including neurodegenerative and inflammatory diseases.

Calpain inhibitors, including peptide aldehydes and α-mercapto-acrylic acid derivatives, are widely used as a valuable tool to elucidate the physiological and pathological roles of calpain. The biological effects of potent calpain inhibitors on human phagocytes (neutrophils and monocytes) are unique, and the inhibitors induce active migration (chemotaxis) of human phagocytes and delay neutrophil apoptosis with rapid activation of the signaling pathways, including Rac, mitogen-activated protein kinases, and phosphatidylinositol 3-kinase. Potent calpain inhibitors have been shown to activate human formyl peptide receptors and/or other G-protein coupled receptors in addition to inhibition of the calpain activity, which may be responsible for the characteristic effects of calpain inhibitors on human phagocyte functions.

Chapter VI – Leaching of toxic substances from the matrix of antifouling paints affects not only the fouling organisms but also "non-target" biota. *Artemia* nauplii have been found to be suitable test organisms for measurement of acute toxicity effects of certain antifouling paints. The present study addresses the impact of a self polishing copper antifouling paint on the activity of the brine shrimp aldehyde dehydrogenase (ALDH). Total ALDH activities from nauplii exposed to several sublethal concentrations ranging from $(S/V)_{50}$ to $1/4(S/V)_{50}$ were determined. Decreased enzymatic activities were observed in all antifouling paint concentrations tested, ranging from 2.4% inhibition at $1/4(S/V)_{50}$ to 53.9% at $(S/V)_{50}$. The results of this preliminary investigation suggest that ALDH activity of *Artemia* nauplii could be used as a biomarker for the evaluation of toxic activity of antifouling paint on non target organisms.