PREFACE

This book presents new developments in proteomics research. Topics discussed include the serine proteinase inhibitor Z Alpha-1 antitrypsin; the structure of Alpha-1 proteinase inhibitor polymer; the roles of mammalian mitogen-activated protein kinase activating protein kinases in cell cycle control and the study of folding/unfolding kinetics of lattice proteins by applying a simple statistical mechanical model for protein folding.

Chapter 1- The serine proteinase inhibitors Alpha-1 Antitrypsin (A1AT) and its mutant protein the so-called 'Z' mutation in A1AT deficiency encodes a glutamic acid-to-lysine substitution at position 342 in A1AT and is the most common A1AT allele associated with disease. In recent years, several important research advances in the cell biology of this aggregation-prone serine proteinase inhibitors and its pathobiological mechanisms for disease onset have proven paramount to our understanding of Z A1AT deficiency. The liver disease associated with Z A1AT, occurs in up to 15% of A1AT-deficient individuals, a result of toxic gain-of-function mutations in the A1AT gene, which cause the A1AT protein to fold aberrantly and accumulate in the endoplasmic reticulum of hepatocytes. The lung disease is associated with loss-of-function, specifically decreased anti-protease protection on the airway epithelial surface. Most strikingly, this polymer has now been revealed to activate the NF-kappaB (NFκB) system and cause cytotoxicity by a pathway that is independent of the unfolded protein response (UPR). NFκB which is an inducible transcription factor which regulates the expression of a range of genes involved in important biological processes such as the innate and adaptive immunity, inflammation, cellular stress responses, cell adhesion, apoptosis and proliferation. This chapter addresses the structural basis of this serine proteinase inhibitor (Z A1AT) and how the ordered accumulation of this polymer activates the NFκB system affecting cell death. Furthermore, we place these findings in the context of the histone code in order shed light on how to target the NFκB system in a specific fashion for the setting of Z A1AT disease.

Chapter 2- The metastable structure of a serpin α_1-proteinase inhibitor (α_1-PI) makes it prone to conformational changes as a result of certain mutations and under mild denaturing conditions. Polymers of several variants of α_1-PI, e.g., the most clinically relevant Z variant, accumulate in the liver and are believed to be the underlying cause of the liver disease associated with α_1-PI deficiency. Such polymers have also been found in the circulation and in the lung. The structure of these polymers, which is important for structure-based drug design, remains unknown. The historically first and generally accepted model of the α_1-PI

polymers, the loop-A sheet model, which assumes insertion of the reactive center loop (RCL) of one α_1-PI molecule between the central strands of the A β-sheet of another molecule, has never been proven. In addition, this model is inconsistent with resonance energy transfer data obtained for heteropolymers formed from the Z and S variants. It is also difficult to envision how this model or even the more compact loop-A sheet model, deduced from fluorescence data obtained for the Z and S variant heteropolymers, can be compatible with electron microscopy (EM) images, which show apparently flexible polymer chains. Two other polymer models were deduced from the interactions seen between molecules in the crystals of two other serpins, antithrombin (AT) and plasminogen activator inhibitor-1 (PAI-1), which demonstrated the ability of the reactive center loop (RCL) to assume the β-strand conformation and to extend the C or A β-sheet in a neighboring molecule by formation of an additional edge strand. This became the ground for the loop-C sheet and strand s7A models of the polymer, although the interactions seen in crystals appear not to be stable in solution and, thus, appear not to reflect interactions existing in α_1-PI polymers, which actually are stable. The focus of this paper is to review the observations used to support the proposed models and to revisit the wealth of literature data in search for unexplained observations and possible new interpretations. I also put forward the hypothesis that a fusion of β-sheets, rather than insertion of the reactive center loop into a β-sheet, may underlie the polymerization process of α_1-PI. This hypothesis is an expansion of the recent head-to-head model proposed based on the polymerization of the α_1-PI disulfide-linked dimer.

Chapter 3- Signal transduction pathways often modulate cell proliferation by targeting the activity of cell cycle regulating proteins. One of the signaling pathways that can control the cell cycle is the mitogen-activated protein kinase (MAPK) signaling cascade. In mammalian cells, the classical MAPK pathway consists of sequential phosphorylation events leading to activation of a MAPK kinase kinase, a MAPK kinase, and a MAPK. MAPK in turn phosphorylates non-protein kinase substrates such as transcription factors, but it can also phosphorylate yet other protein kinases, referred to as MAPK-activating protein kinases (MAPKAPK). Eleven mammalian MAPKAPKs have been identified so far; six of them belong to the group of AGC protein kinases (RSK1, RSK2, RSK3, RSK4, MSK1, and MSK2), while the other five belong to the family of calmodulin-dependent kinases (MK2, MK3, MK5, MNK1, and MNK2). In this review we will discuss those MAPKAPKs that play a role cell-cycle regulation as well as the potential use of specific MAPKAPK inhibitors as therapy in conditions with abnormal cell cycle regulation.

Chapter 4- The folding/unfolding kinetics of a three-dimensional lattice protein was studied using a simple statistical mechanical model for protein folding that we had developed earlier. The model considers the specificity of an amino acid sequence and the native structure of a given protein. We calculated the characteristic relaxation rate on the free energy surface starting from a completely unfolded structure (or native structure) that is assumed to associate with a folding rate (or an unfolding rate). The chevron plot of these rates as a function of the inverse temperature was obtained for four lattice proteins, *a1*, *a2*, *b1*, and *b2*, in order to investigate the dependency of the folding and unfolding rates on their native structures and amino acid sequences. Proteins *a1* and *a2* fold to the same native structure, but their amino acid sequences differ. The same is true for proteins *b1* and *b2*, but their native structure is different from that of *a1* and *a2*. To elucidate the roles of individual amino acid residues in protein folding/unfolding kinetics, we calculated the kinetic properties for all

possible single amino acid substitutions of these proteins and examined their responses. The results are discussed with respect to the roles of short- and long-range interactions and formation of a folding nucleus in the kinetics of protein folding/unfolding.

Chapter 5- The interest in intrinsically unordered proteins (IUPs) has greatly increased, as it has become clear that they are very widespread, especially in eukaryotic organisms. The presence of unordered regions in functional proteins is implicated in important biological roles, such as translation and transcriptional regulation, cell signaling and molecular recognition. A number of studies report that for mammals about 75% of their signalling proteins are predicted to contain long unordered regions (>30 residues), about half of their total proteins are predicted to contain such long unordered regions, and about 25% of their proteins are predicted to be fully unordered. Other studies report examples of unordered proteins implicated in important cellular processes, undergoing transitions to more structured states upon binding to their target ligands, DNA, or other proteins. The accumulation of such evidence led to the suggestion that the classical protein structure-function paradigm needs to be reassessed. In fact, the assumption that a folded three-dimensional structure is necessary for function has been modified. Although the functions of many proteins are directly related to their three-dimensional structures, numerous proteins that lack intrinsic globular structure under physiological conditions are now recognized. As the amino acid sequence contains the information for protein folding, it was reasoned that, also for proteins that do not fold into three-dimensional structures, the residue sequence should specify protein "nonfolding". Therefore, to test this hypothesis, a large number of methods were developed to predict regions of protein sequences that fail to fold. Their prediction accuracy has been better than expected and this suggests that the information for failure to fold into a regular structure is likely to be inherent within the residue sequence. Because there is an enormous interest in predicting unordered regions of proteins, the analysis of the possibility of predicting protein "unordered" regions was addressed also in the last four editions of CASP experiment.

The main aim of this chapter is to present the state of the art of prediction methods of protein unordered regions developed in the last years and their application to specific protein families. In particular, we will focus our discussion on human Sirt-1 and cytokine membrane receptors because our data suggest that these proteins, involved in chronic inflammatory diseases, have unordered structural segments very important for their function.

Chapter 6- The 'foldase' enzyme protein disulphide isomerase (PDI) interacts with nascent polypeptides in the lumen of the endoplasmic reticulum to catalyze the formation of new disulphide bonds, as well as breakage and alternative isomerization of incorrectly formed bonds, during protein folding and maturation processes in eukaryotic cells. PDI belongs to the thioredoxin (TRX) superfamily that catalyzes cellular redox reactions and has an active site with the conserved motif WCXXC. PDI is a member of the larger PDI-like (PDIL) protein family, its subclasses varying in numbers, positions and sequences of functional domains and active sites and in subcellular locations and functions, many including PDI also playing chaperone roles. The human PDIL family has been studied in great detail, its members exhibiting activities ranging from the archetypical disulphide formation/isomerisation to a multitude of chaperone roles of relevance to cell biology and medicine, for example, in neurodegenerative diseases, apoptosis and cancer. The plant PDIL families are larger and more diverse and have been associated with some unique roles such as storage protein folding; however, information on plant PDILs is much more limited. This work overviews the key properties and functions of human PDILs, summarises the diversity and known functions

of plant PDILs, and contemplates the directions for future research on PDILs, particularly in plants.

Chapter 7- Many organisms grow in extreme environments on Earth. Microorganisms whose optimal growth temperature is above 80°C are called hyperthermophiles. Proteins from hyperthermophiles usually exhibit higher stability than those from organisms that grow at lower temperatures. In this paper, we describe our studies of the stability and folding of ribonuclease HII from the hyperthermophile *Thermococcus kodakaraensis* and introduce other studies of hyperthermophilic proteins. These studies focus on hyperthermophilic proteins with reversible unfolding and examine their equilibrium and kinetic aspects. The results indicate that kinetic stability (slow unfolding) is a general strategy by which hyperthermophilic proteins adapt to higher temperatures. Mutational analysis indicates that hydrophobic interaction is a factor in the molecular mechanism of the slow unfolding of hyperthermophilic proteins. Next, we report on studies of subtilisin-like serine protease from *Thermococcus kodakaraensis*. The results demonstrate that this protein is extremely stable and that the maturation (folding) process of this protein differs from those of mesophilic homologues in terms of the role of Ca^{2+} and propeptide. This protein is thought to be stabilized due to a high kinetic barrier between the unfolded and folded states. To reduce this barrier, the folding may be fine-tuned by utilizing Ca^{2+} and propeptide. We discuss protein folding from the viewpoint of adaptation to hot environments.

Chapter 8- One of the central characteristics of a living system is the ability to self-assemble its component molecular structures with precision and fidelity. The folding of proteins into their compact three-dimensional structures is the most fundamental and universal example of biological self-assembly. Understanding this complex process will therefore make available a unique insight into the way in which evolutionary selection has influenced the properties of a molecular system for functional advantage. The final goal of folding studies is to predict structure from sequence, allowing the design of new functional proteins and prevention of abnormal disease-associated protein conformations.

Chapter 9- A protein kinase is an enzyme that modifies other proteins by chemically adding phosphate groups to them (phosphorylation). Phosphorylation usually results in a functional change of the target protein (substrate) by changing enzyme activity, cellular location, or association with other proteins. Protein tyrosine kinases (PTKs) play a key role in the regulation of cell proliferation, differentiation, metabolism, migration, and survival. Due to their involvement in various forms of cancers, PTKs have become prominent targets for therapy. There are two principles to developing PTK inhibitors. As binding APT is essential for kinase activity, the common strategy is to look for the small molecules that can compete the ATP binding sites. To increase specificity of PTK inhibitors, a more attractive strategy is to develop non-ATP-competitive kiniase inhibitors. So far, there are many PTK inhibitors that have been developed. Several inhibitors have been successfully used to treat human cancers in clinic. These agents are shown to inhibit multiple functions of cancer cells, including proliferation, survival, invasion, and angiogenesis.

Chapter 10- Treatment options for cancer have increased in the last years due to the ongoing research and development of new therapeutics. A focus shift from formerly undirected cytostatic agents to more targeted therapies, mainly antibodies and protein kinase inhibitors has taken place. New agents directed against receptors which control proliferation, migration and angiogenesis of tumor cells have been developed. Mainly specific antibodies directed against the extracellular ligand binding domain of various receptor tyrosine kinases,

and protein kinase inhibitors targeting the intracellular protein kinase domain of receptors or cytoplasmic signalling proteins have been increasingly developed in the past years. In this chapter we will address the current treatment options with protein kinase inhibitors exemplarily for hepatocellular carcinoma and renal cell cancer. We will further give an insight into the actual research perspectives and the conception of incorporating new drugs into clinical trials as single agent treatment or combined with "classical" chemotherapy. The first part elucidates the molecular pathways of carcinogenesis and depicts the mode of action of these new agents in the treatment of hepatocellular carcinoma, the second part illustrates the pathogenesis of renal cell cancer and the resulting therapeutic options by targeting these pathways.