PREFACE

Antifreeze proteins, also known as thermal hysteresis proteins, ice binding proteins and ice structuring proteins, prevent the growth of ice crystals in several cold blooded organisms. First discovered in fish, they have also been found in insects, plants, fungi and bacteria. Antifreeze proteins cause the non-colligative depression of the freezing point of water, a property which has been exploited in the practical applications of antifreeze proteins such as improving the texture of ice cream, and could be used to extend the crop growing season or allow fish to thrive in cold waters. This book provides clear information on what is known about antifreeze proteins today and how to study them.

Chapter I - Robert Earl Feeney was, in 1964, in his own words, "a contented, middle-aged professor, complete with gray hair, a slight stoop, glasses to peer over, and a well-equipped laboratory". In his well-established position, well-funded by the National Institutes of Health, he was engaged in research to unlock the secrets of why certain animals can be so similar in many ways and still have biochemical characteristics that are widely different. The hypothesis he held was that the egg-white proteins hold many of these secrets. One important variation is that of living organisms' different tolerances to cold and warm environments. So from 1964 into the 1970's, Robert E. Feeney became an explorer in the wilds of the Antarctica Continent and its surrounding icy seas. Ostensibly the initial mission was to obtain and compare the egg-white from the cold-region adapted penguins to birds from the more temperate regions of the world. His work on egg white proteins required him to obtain penguin eggs, and that started his field trips to the Antarctic in 1964. Once there, he rapidly became absorbed in the marvels of how certain species of fish survive in the sub-zero Antarctic sea, where temperature is almost constantly near $-1.84^{\circ}C$.

Chapter II - When determining antifreeze activity one of the biggest issues over the past couple of decades has been how to compare results from various laboratories, where different instruments and/or procedures are used. Some of the reasons for this are historical, while others come from the fact that, even though there are many complicated techniques available, experienced scientists can apply simplified and more general techniques which still provide valuable and reliable measurements of antifreeze activity.

Chapter III - With about 80% of the earth's surface climate 15°C or lower on average, animals have adapted various strategies to subsist. Few organisms can maintain a sufficiently active metabolism at very low temperatures and have adopted various behavioural strategies for survival. Some animals such as birds, mammals and butterflies migrate seasonally to warmer climates to avoid the effects of low temperatures. Other animals such as bears, bats, frogs and snakes hibernate underground and reduce their metabolism. Many insects overwinter arrested in various stages of development, hatching and growing in the summer. Plants have adapted similar overwintering strategies of arrested development with many plants dying back, keeping only their root system alive.

Non-migratory animals, microorganisms, plants and insects that cannot escape low temperatures must use a different strategy for survival. Freezing can be avoided by producing high quantities of antifreezes such as glycerol and sorbitol to lower the supercooling point, sometimes in conjunction with antifreeze proteins (AFPs) that bind to embryonic ice crystals preventing further, potentially damaging, crystal growth; such a strategy is used by several insects and plants.

Chapter IV - Ice nucleation is the process that initiates the conversion of liquid water to the solid form, ice. Nucleation requires that the water be *supercooled*, i.e. in a liquid state at a temperature below the equilibrium freezing point or melting point. Ice nucleation may originate from the water molecules themselves, which tend to form larger aggregates when the temperature drops. When an aggregate reaches a certain critical size, ice will spread from this nucleus throughout the sample. When ice formation is initiated in this way, i.e. from a nucleus formed by the water molecules themselves, it is referred to as *homogeneous* nucleation.

The presence of other molecules or particles in the supercooled water may catalyze the formation of a critically large water molecule aggregate, and thus trigger nucleation at a temperature higher than that where the water molecules alone could initiate freezing. When ice formation is catalyzed by other structures than the water molecules themselves, it is referred to as *heterogeneous* nucleation. These nucleating structures must somehow have the capacity to organize the water molecules in an ice-like fashion, and in aggregates larger than those which otherwise exist in the water sample at that temperature.

Chapter V - Water is of fundamental importance to life on earth. Nearly two-thirds of the surface of the earth is comprised of water, with the average surface temperature of seas and oceans varying from -2°C to +30°C depending on latitude. Within the Polar Regions, seawater temperatures are consistently below the freezing point of physiological solutions, which themselves have freezing points below the freezing point of pure water (0°C at 1 atmosphere), due to dissolved sugars and salts. Adaptation schemes for survival at these extreme environments have resulted in the evolution of unique protein super families that permit organisms to exist and thrive over a broad geographic distribution. This class of proteins, defined by antifreeze proteins, exists in low-temperature environments to inhibit lethal intra- or extracellular ice formation. After the initial reports of these proteins by Scholander and co-workers, DeVries and Feeney were the first to investigate the mechanisms of antifreeze action. Antifreeze proteins (AFPs) and their glycosylated forms, antifreeze glycoproteins (AFGPs), also known as thermal hysteresis proteins, have since been identified in the body fluids and tissues of many species of polar fish.

Chapter VI - The power of structural biology lies in its ability to explain how proteins function at the atomic level. The 3D structures of antifreeze proteins (AFPs) have been studied for over 20 years, and author have gained an incredible amount of knowledge about them. Author are still, however, searching for a consistent model that will explain how all AFPs work because there is no universal sequence or structural motif. As Figure 1 illustrates, AFPs from fish, insects, and plants show no obvious conserved structure. If anything, it appears that with the identification of new AFPs, even more new sequences and folds are described. This lack of structural conservation has been pushed even further with the discovery that dehydrins, plant proteins that are intrinsically disordered, can have antifreeze protein activity.

Discovering a common motif has been complicated by the fact that AFPs have a very unusual ligand – ice – which makes it difficult to determine ligand-bound forms using routine biophysical methods. This chapter describes the structures and ice-binding residues of several AFPs; following that will be a similar analysis of two theoretical plant AFP structures that have been examined mainly by homology modeling. The descriptions will also include information on the dynamics, water-binding characteristics and other interesting aspects of these proteins. The chapter will conclude with a look at the future – the full structural determination of AFPs in ice, in the hopes that author may really understand how these proteins bind to their ligand.

Chapter VII - The field of antifreeze (glyco)proteins, AF(G)P, began with the observation that fish in the Antarctic waters survive at -1.9°C. DeVries and Wohlschlag followed with a report of the first discovery of AFGPs that inhibit ice growth. The unusual property exhibited by the AF(G)Ps is a phenomenon known as thermal hysteresis, the difference between the temperatures at which a AF(G)P solution freezes and melts. It has been suggested that perhaps a more appropriate name for these proteins would be thermal hysteresis proteins (THP); however, in this chapter author will continue to use the AF(G)P designation to be consistent with the literature. AF(G)Ps are widely found in bacteria, fish, insects, and plants.

The interest in understanding the behaviors of these proteins, and more importantly their novel physical properties, is driven by the impact this knowledge could have on biotechnology. The food industry has made use of the properties of AFPs to improve ice cream quality. The fishery industry is exploring the incorporation of the AF(G)P gene into fish for cold-climate fish farms with longer growing seasons. The agricultural field is looking into the use of AF(G)Ps in citrus crops. The aviation industry has investigated the use of AF(G)P-like compounds in de-icing of aircraft and in aircraft fuel. The ability to lower organ temperatures for transport and the identification of a cryo-protectant useful for blood storage are of medical interest. Since it is well-known that AF(G)Ps can inhibit ice crystal growth, scientists have investigated the use of AFPs in biomineralization processes such as the crystal growth of materials like calcite.