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# Biophysical and biochemical aspects of antifreeze proteins: Using computational tools to extract atomistic information

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ABSTRACT

Antifreeze proteins (AFPs) are the key biomolecules that protect species from extreme climatic conditions. Studies of AFPs, which are based on recognition of ice plane and structural motifs, have provided vital information that point towards the mechanism responsible for executing antifreeze activity. Importantly, the use of experimental techniques has revealed key information for AFPs, but the exact microscopic details are still not well understood, which limits the application and design of novel antifreeze agents. The present review focuses on the importance of computational tools for investigating (i) molecular properties, (ii) structure–function relationships, and (iii) AFP-ice interactions at atomistic levels. In this context, important details pertaining to the methodological approaches used in molecular dynamics studies of AFPs are also discussed. It is hoped that the information presented herein is helpful for enriching our knowledge of antifreeze properties, which can potentially pave the way for the successful design of novel antifreeze biomolecular agents.



## 1. Introduction

Antifreeze proteins (AFPs) are the key biomolecules that are responsible for the survival of species in extreme climatic conditions (Davies and Hew, 1990). The emergence of these biomolecules in species is specially adapted through natural selection for survival (Logsdon and Doolittle, 1997). Freezing can be defined as the condition when the temperature approaches at, or below, the freezing point for the water to release sufficient heat and become solid (ice). In terms of physical chemistry, slight-under-cooling condition for the solution below the equilibrium freezing point results in freezing (Knight et al., 1991). This phenomenal event includes specialized rearrangement of randomly oriented (anomaly) water molecules into a uniform arrangement of ice crystals (Clark and Worland, 2008). AFPs are known to get adsorbed onto the ice surface at one or more specific direction depending upon their properties and types (Davies and Sykes, 1997). The adsorption further leads to the retardation of successive growth of ice lattice, which in turn results in creating micro-curvature formation. This underlying mechanism is more precisely denoted as "lowering of freezing point" of water in a non-colligative manner (Knight et al., 1991), more explicitly as the "Gibbs–Thomson Kelvin effect". Lowering of the freezing point of water by AFPs is thus mediated without affecting the melting point (Chapsky and Rubinsky, 1997).

Over the past decades, significant investigations of the underlying mechanism of action in terms of sequential context and structural motif of AFPs have been reported (Atici and Nalbantoglu, 2003; Chao et al., 1997; Dalal and Sönnichsen, 2000). Such information includes the crucial roles played by several amino acids (e.g., Ala, Thr, Val) in the interaction phenomenon with ice-crystal (Deng et al., 1997). Likewise, the contribution from van der Waals and electrostatic interaction by Asn and Leu are also accounted for finding the possible explanation (Davies and Hew, 1990; Deng et al., 1997). These studies suggest that the flexibility of hydrophobic residues side chains and the entropy-driven process of ice binding are the key determinants that govern the ice binding nature of these biomolecules (Jia and Davies, 2002).

Based on the structural arrangement and varying properties, AFPs have been categorized mainly into five types: Type I, II, III, IV, and antifreeze glycoproteins (AFGPs) (Davies and Sykes, 1997; Venketesh and Dayananda, 2008). Type I AFPs have the distinct alpha-helical straight geometry of 3–4 kDa. Type II AFPs, on the other hand, are globular proteins having a minimum of five disulfide bonds; these protein structures mainly range from 11.3 to 24 kDa. Likewise, Type III AFPs are characterized by beta-clips in the globular proteins, with an average molecular weight of 6.5 kDa.

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Type IV AFPs exhibit bundles of alpha-helical protein rich in amino acids, such as glutamate and glutamine, with a molecular weight of 12 kDa (Rosa and Franz, 1999). Among all these types, the relevant information pertaining to rigorous studies of AFPs are available with respect to type I AFPs, isolated from winter flounder, sculpin, and other relative organisms (Evans and Fletcher, 2005). On the other hand, sparse information is available in the literature on the structure of type IV AFPs. A cartoon representation of various types of AFPs is shown in Fig. 1.

In light of the above, it is clear that structural information is strongly correlated to biological relevance (Davies, 2014; Davies et al., 2002) Remarkable properties of molecular recognition are thus helpful in framing assumptions either to support the experimental results or to fill the informational gap difficult to fill with only experimental tools (Madura et al., 2000). In this regard, computational tools are particularly helpful for obtaining in-depth microscopic structural and functional insights at atomistic level. The properties pertaining to antifreeze activity of AFPs can be potentially characterized by thermal hysteresis and ice recrystallization assays (Drori et al., 2014; Xiao et al., 2014). Once it is confirmed that the biomolecule possesses activity relevant in physiological terms for the survival of species in the extreme weather conditions, it becomes convenient to identify the structural motifs and arrangement of amino acids. Structural and functional information of AFPs are reported mainly using high and low-resolution spectroscopy techniques. Circular dichroism spectroscopy and infra-red spectroscopy are useful to identify the secondary structural characteristics (Shah et al., 2012). Similarly, the structural elucidation in terms of globular packing and architectures are reported using X-ray and NMR techniques (Pentelute et al., 2008; Sönnichsen et al., 1998). The use of microscopic tools, such as ice crystallization inhibition assay and ice microscopy, also confirms the relative retardation in growth of ice crystals (Drori et al., 2014; Shah et al., 2012). Along with the beneficial aspects of these experimental techniques, there exist certain limitations, which fail to explain some of the functional attributes of AFPs. The exact dynamical features between AFPs and ice, role of van der Waal and electrostatic contribution, role of polar and hydrophobic interaction types, and steric factors that contribute to the overall interaction are yet to be completely elucidated. Similarly, the use of bioinformatics tools for tracing the evolutionary relationships and prediction of engineered peptides for requisite activity are difficult or time consuming to study with experimental techniques. Thus, the usage of other similar techniques related to theoretical or computational tools along with the experimental techniques are important to elucidate the desired in-depth information. The employment of a combined approach involving both experimental and computation techniques in AFPs studies is thus crucial. It not only helps in identifying the key structural features involved in the interaction, but also assists in tracing features that may prove worthy in designing novel biomolecules with comparable antifreeze activity. Considering these aspects, the present review focuses on the feasibility of computational tools and techniques for extracting information related to AFPs (Nada and Furukawa, 2012), both in structural terms as well as in methodological prospect. We believe that such information is timely and essential for a better understanding of the biophysical and biochemical properties of AFPs.

## 2. Antifreeze proteins (AFPs)

Experimental and theoretical studies have conferred that antifreeze activity executed by AFPs are mediated by virtue of strong interaction between antifreeze proteins and ice structure (Ebbinghaus et al., 2012). Several studies suggest that a strong and stable binding of AFPs with ice crystals along specified plane is essential (Sönnichsen et al., 1995). In this context, it is vital to understand the phenomenon of growth of ice crystals at the molecular scale. Additionally, different structural motifs and relative arrangements of residues in the globular packing contribute to this interaction as well (Wen and Laursen, 1992). Although these pieces of information are possible to obtain from experimental methods, the exact mechanisms governing these phenomena remain ambiguous to date (Sharp, 2011). According to some studies, the mechanism of interaction between AFPs and ice structure is mediated via hydrogen bonding or polar interaction (Dalal et al., 2001), while van der Waals interaction between residues and voids may be prudent in this regard, as suggested in other instances (Jorov et al., 2004). Thus, the overall mechanism of interaction and action is still, to date, the subject of considerable debate.

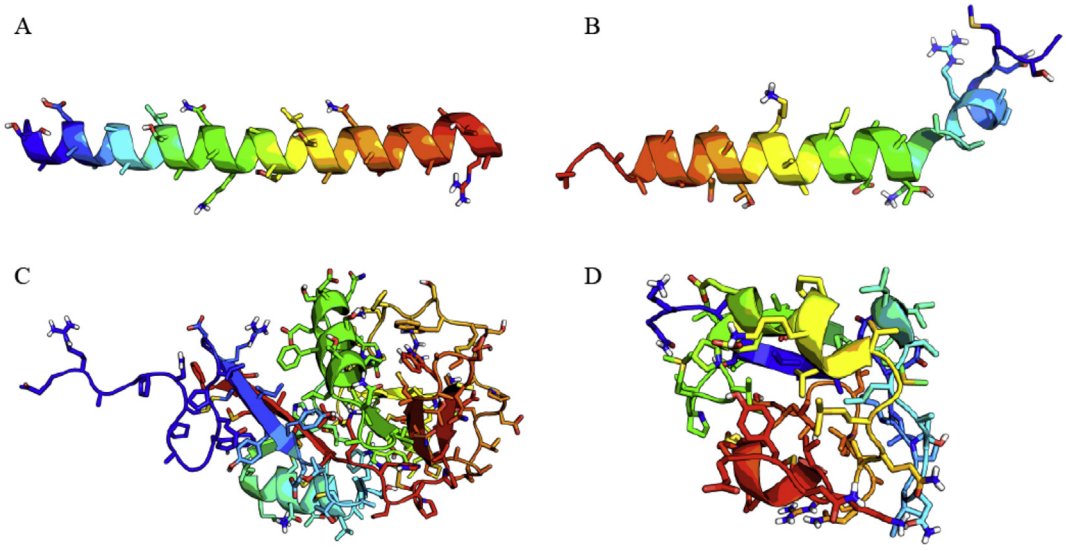


**Fig. 1.** Cartoon representation of various AFPs available in protein data bank. PDB accession code of the structures are (A) type I, winter flounder (1WFA); (B) type I, shorthorn sculpin (1Y03); (C) type II, sea raven (2AFP); and (D) type III, eel pout (1UCS).