Anchored clathrate water hypothesis explains how proteins recognize and bind ice.

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Antifreeze proteins (AFPs) all have an ice-binding site (IBS) – an extensive surface that selectively binds the protein to one or more planes of ice, and which can be disrupted by surface amino acid replacements. The IBS is typically the most hydrophobic surface of the AFP, with few if any charged groups. It is often structurally repetitive and relatively flat. Based on the crystal structure of a bacterial AFP (Garnham *et al.* 2011), and other evidence emerging in the literature, we have argued that the IBS organizes water around hydrophobic groups into an ice-like clathrate structure that is anchored by hydrogen bonds to backbone and or side-chain groups on the protein. In essence, the AFP forms the ligand to which it then binds through a merger of the ice-like waters on the IBS and the quasi-liquid layer surrounding ice, as suggested by Nutt and Smith (2008). The anchoring of the clathrate waters incorporates elements of the original hydrogen-bonding hypothesis and the hydrophobic effect mechanism. However, the most effective anchoring would come from hydrogen bonding groups that are buried in the hydrophobic surface; and the role of the hydrophobic surface is to constrain the waters into an ice-like pattern for the merger rather than for entropic gain when released from the surface on AFP binding to ice.

Here we have reviewed previously solved AFP crystal structures in the database to look for anchored clathrate waters. In most of these structures the IBS, being extensive, flat, and relatively hydrophobic, has served as a main protein-protein crystal contact, leaving few surface-bound waters (or none in the case of type I) in position. Nevertheless, the remaining waters generally take up ice-like positions. Furthermore, using the TIP5 water model in molecular dynamics calculations, missing waters occupy their expected ice-like positions on the IBSs. The anchored clathrate waters will only have slightly longer surface residence times on the IBS than other waters around the protein, and the AFP will only bind to ice in the instant when a quorum of these waters is in place. This dynamic can help explain the dramatic increase in thermal hysteresis activity that results when the ice-binding site is made larger. The larger the IBS the more likely a quorum of ice-like waters will form. It also helps explain the dependence of thermal hysteresis (TH) on AFP concentration and cooling rate, because both factors increase the availability of binding-competent AFPs. At any one time the vast majority of AFPs in solution will not have a quorum present. Surface mutants that decrease TH need to be reinterpreted in the light of what these amino acid changes might do to the number of clathrate waters on the IBS. We predict the mutations will decrease the number of ice-like waters on the IBS, which again is consistent with the observation that it is possible to compensate for the decrease in TH of mild AFP mutants by increasing their concentration. Our modeling studies (see poster by Rob Campbell *et al.*) suggest that ice-nucleation proteins also function by organizing anchored clathrate waters, but on a much larger scale. Because INPs are much larger than AFP and can oligomerize they bring enough ice-like waters together to begin nucleation at high sub-zero temperatures.

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