Antifreeze Protein Scaffold for the Design of Novel Surface Binding Biomolecules

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Protein scaffolds are increasingly being adapted and engineered to provide molecules with novel activities or binding specificities. The *Tenebrio molitor* thermal hysteresis protein, also known as antifreeze protein (AFP), is a potential scaffold for generation of non-natural proteins that bind to two-dimensional (2D)-surfaces of useful inorganic materials, such as semiconductors. AFP combines the advantages of (i) small size, (ii) stability and rigidity conferred by the conserved eight disulfide bridges and a repetitive beta-helix, and (iii) availability of a 2D face with strong structural periodicity, naturally evolved to bind surfaces of ice crystals. These properties make the TmTHP scaffold beneficial relative to other proteins or peptides, potentially increasing the specificity of surface recognition by minimizing the entropic cost of binding. We developed an efficient protocol for expression and purification of AFP and showed that it is active, stable, and able to recruit fused proteins to ice. To determine the robustness of the AFP scaffold to mutagenesis and therefore its suitability for re-engineering, we constructed a series of mutants with global replacement of the residues involved in ice binding. Each mutant contains an exposed array of a particular amino acid side-chain such that a repetitive arrangement of altered functional groups is displayed on the surface. Structural studies of the mutants (circular dichroism, NMR) show that the AFP fold is retained in most cases despite the comprehensive and severe changes in primary structure and chemical properties. We are developing a phage display system for selection of AFP variants with specific surface binding properties from a combinatorial library. We engineered bacteriophages displaying AFP and showed that AFP is folded on the surface of the phage particle. We constructed a AFP gene library of ~10^7 unique variants with diversified amino acids on the ice binding surface. We intend to custom-evolve novel 2D surface-binding proteins by cycles of selection from the AFP library.