

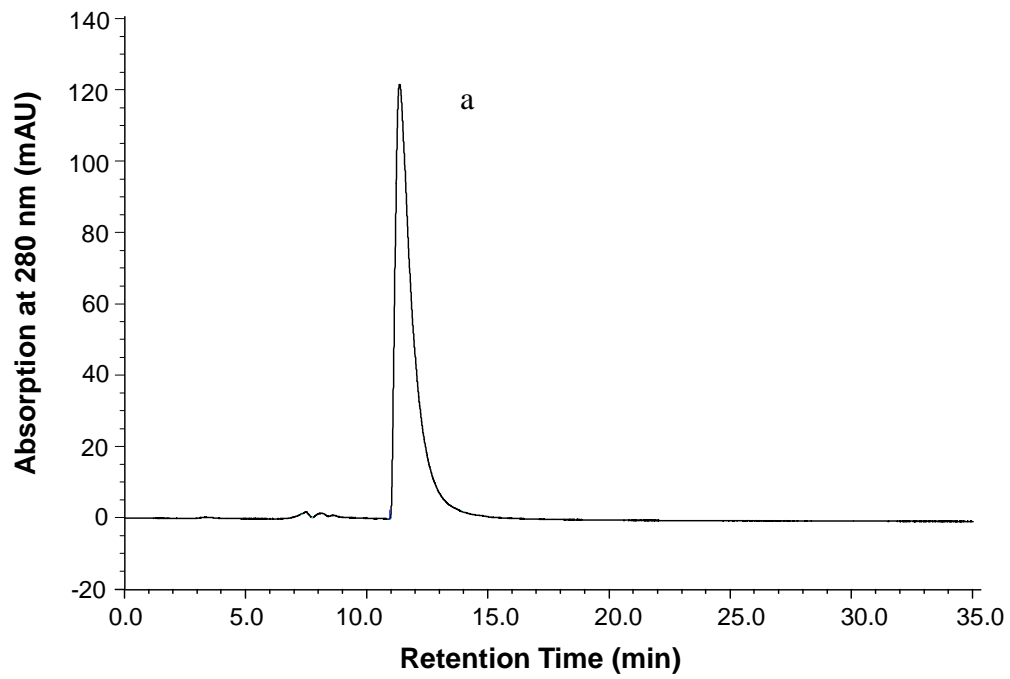
## Supporting Materials

### Arginine, a key residue for the enhancing ability of an antifreeze protein of the beetle *Dendroides canadensis*

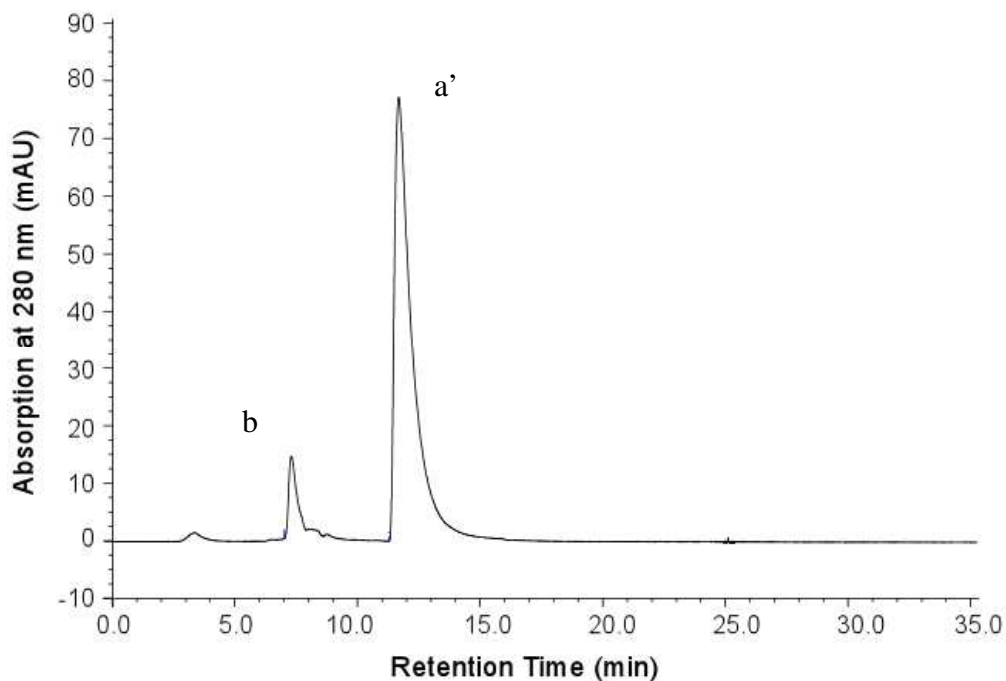
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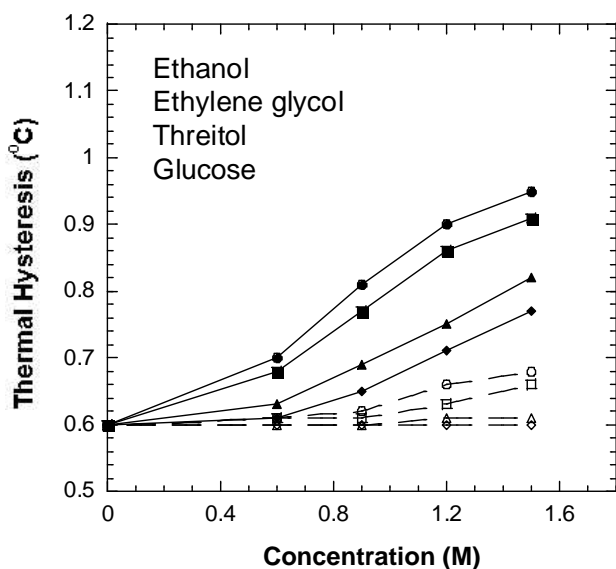
A.



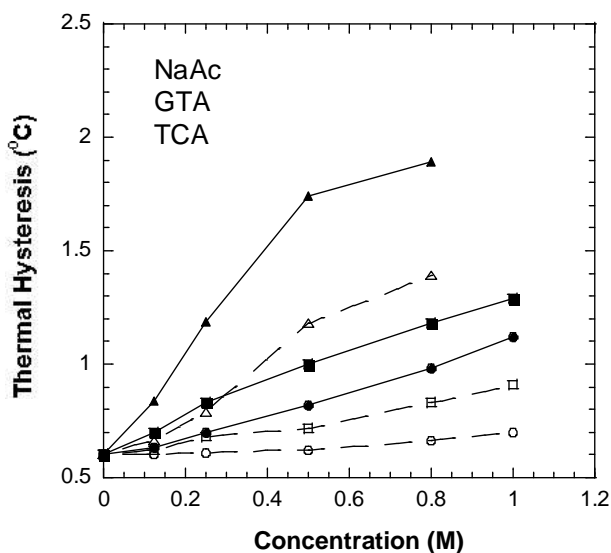
B.



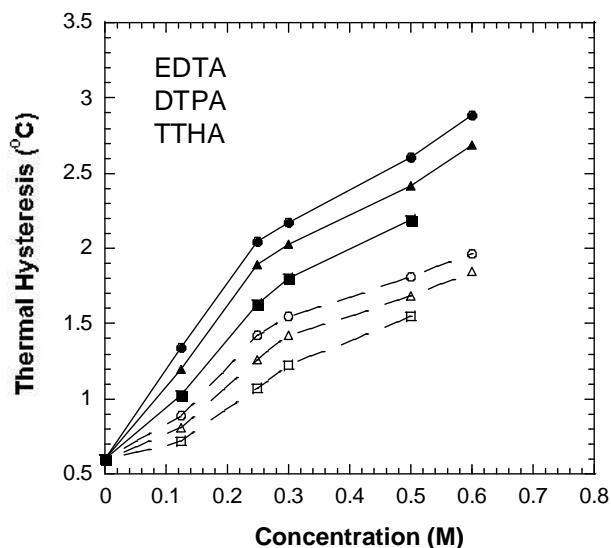
**FIGURE S1.** Quantitative analysis of the modification reaction of DAFP-1. UV absorption detected size exclusion profiles of (A) 1,2-cyclohexanedione (2.970 mM, 8  $\mu$ L,  $t_R$  = 11.5 min); and (B) the reaction mixture of DAFP-1 (0.139 mM, 8  $\mu$ L,  $t_R$  = 7.1 min) and 1,2-cyclohexanedione (1.910 mM, 8  $\mu$ L,  $t_R$  = 11.5 min). The molar ratio of DAFP-1 to 1,2-cyclohexanedione in the reaction mixture was 1:13.7. The area under peak a in (A) is a measure of the amount of 8  $\mu$ L of 2.970 mM 1,2-cyclohexanedione. The area under peak a' in (B) is a measure of the amount of the excess 1,2-cyclohexanedione after the reaction with DAFP-1. The ratio of the area under peak a ( $A_a$  = 99.8 mAu  $\cdot$  min) to the area under peak a' ( $A_{a'}$  = 59.5 mAu  $\cdot$  min) is 1.68, which is the same as the ratio of the amount of 1,2-cyclohexanedione used (2.97 mM, 8  $\mu$ L) to the amount of the excess 1,2-cyclohexanedione after 1:1 reaction with DAFP-1 (1.910 mM – 0.139 mM = 1.771 mM, 8  $\mu$ L).



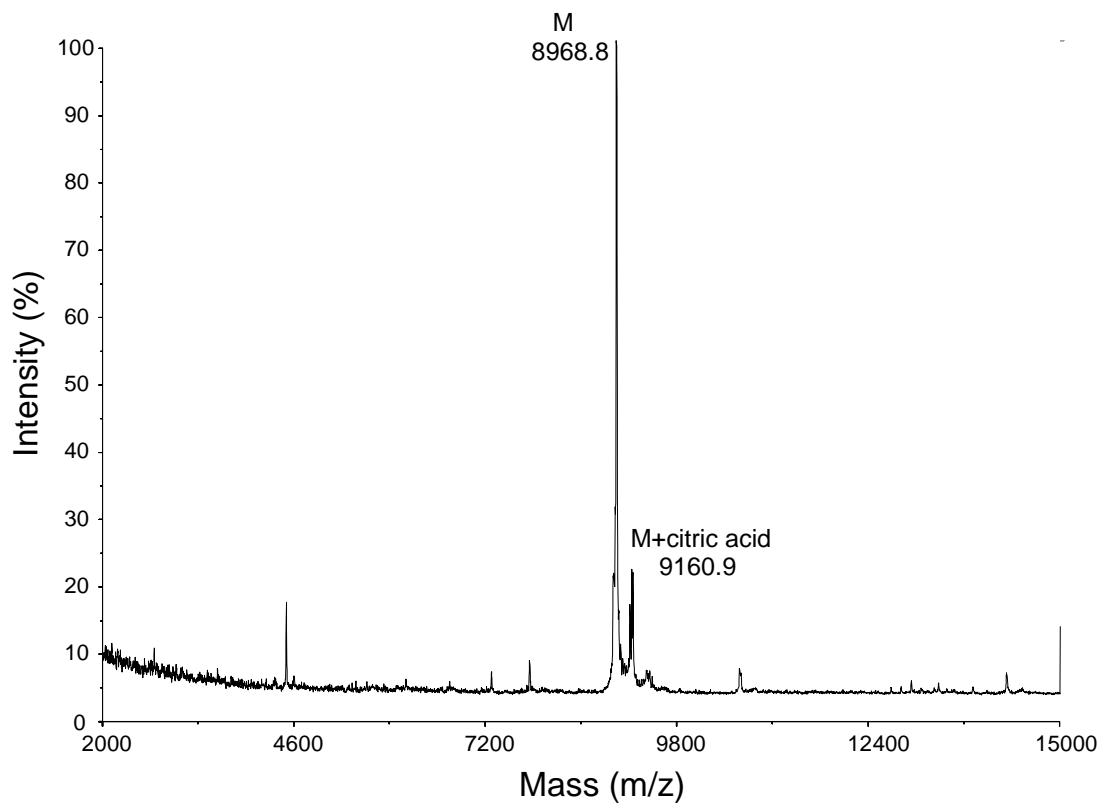
**FIGURE S2.** The antifreeze activities of DAFP-1 and the Arg-modified DAFP-1 in the presence of ethanol ( $\blacklozenge$ ,  $\lozenge$ ), ethylene glycol ( $\blacktriangle$ ,  $\triangle$ ), threitol ( $\blacksquare$ ,  $\square$ ), glucose ( $\bullet$ ,  $\circ$ ), respectively, in 50 mM borate buffer pH 9.0. The filled symbols were used for DAFP-1 and the open symbols were used for the Arg-modified DAFP-1.



**FIGURE S3.** The antifreeze activities of DAFP-1 and the Arg-modified DAFP-1 in the presence of sodium acetate, NaAc ( $\bullet$ ,  $\circ$ ), sodium glutarate ( $\blacksquare$ ,  $\square$ ), and sodium tricarboxylate ( $\blacktriangle$ ,  $\triangle$ ), respectively, in 50 mM borate buffer pH 9.0. The filled symbols were used for DAFP-1 and the open symbols were used for the Arg-modified DAFP-1.



**FIGURE S4.** The antifreeze activities of DAFP-1 and the Arg-modified DAFP-1 in the presence of sodium ethylenediaminetetraacetate, EDTA (■, □), sodium diethylenetriaminepentaacetate, DTPA (▲, △), and sodium triethylenetetramine-*N,N,N',N'',N''',N''''*-hexaacetate, TTHA (●, ○), respectively, in 50 mM borate buffer pH 9.0. The filled symbols were used for DAFP-1 and the open symbols were used for the Arg-modified DAFP-1.



**FIGURE S5.** MALDI-TOF mass spectrum of DAFP-1 incubated with citrate at pH 7.4 in 0.10 M sodium phosphate buffer, pH 9.0. M indicates DAFP-1.