



Title	Concentration-dependent oligomerization of an alpha-helical antifreeze polypeptide makes it hyperactive
Author(s)	Mahatabuddin, Sheikh; Hanada, Yuichi; Nishimiya, Yoshiyuki; Miura, Ai; Kondo, Hidemasa; Davies, Peter L.; Tsuda, Sakae
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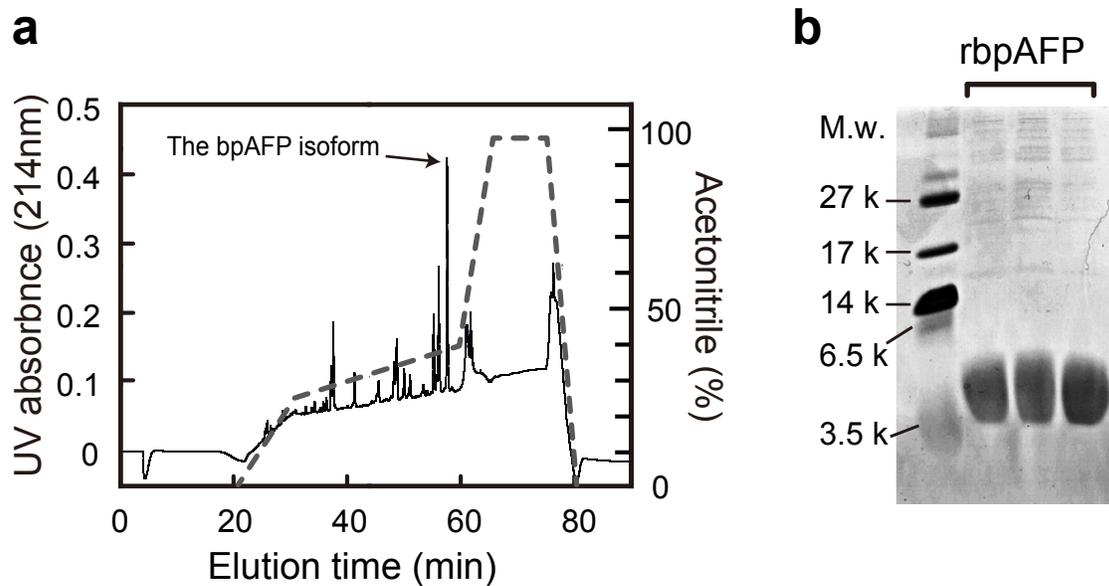
Sheikh Mahatabuddin^a, Yuichi Hanada^a, Yoshiyuki Nishimiya^b, Ai Miura^b, Hidemasa Kondo^{a,b},
Peter L. Davies^c & Sakae Tsuda^{a,b}*

^aGraduate School of Life Science, Hokkaido University, Sapporo 060-0810, Japan.

^bBioproduction Research Institute, National Institute of Advanced Industrial Science and Technology (AIST), Sapporo 062-8517, Japan.

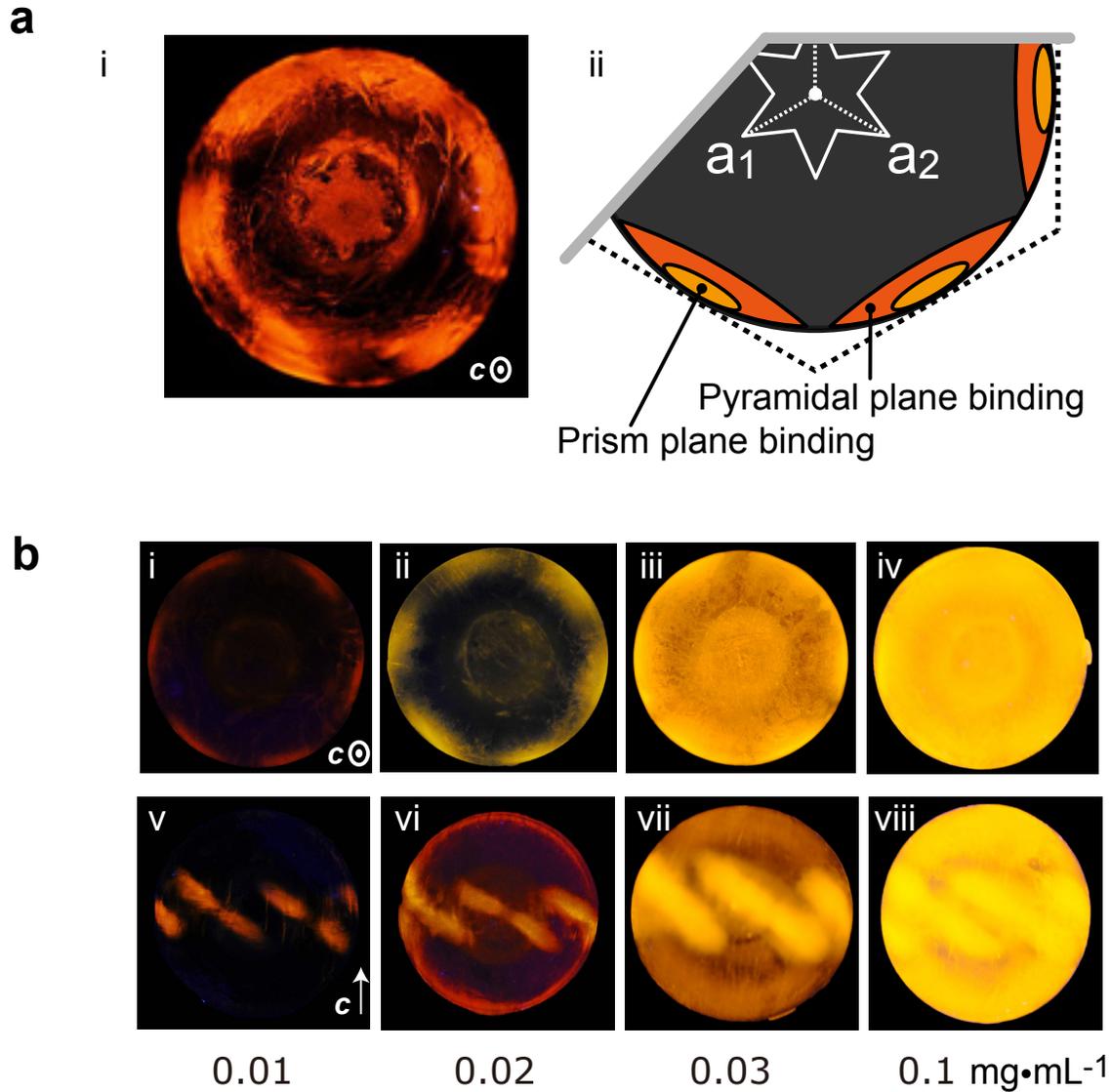
^cProtein Function Discovery Group and Department of Biomedical and Molecular Sciences, Queen's University, Kingston, ON, Canada K7L 3N6.

Supplementary Figure S1



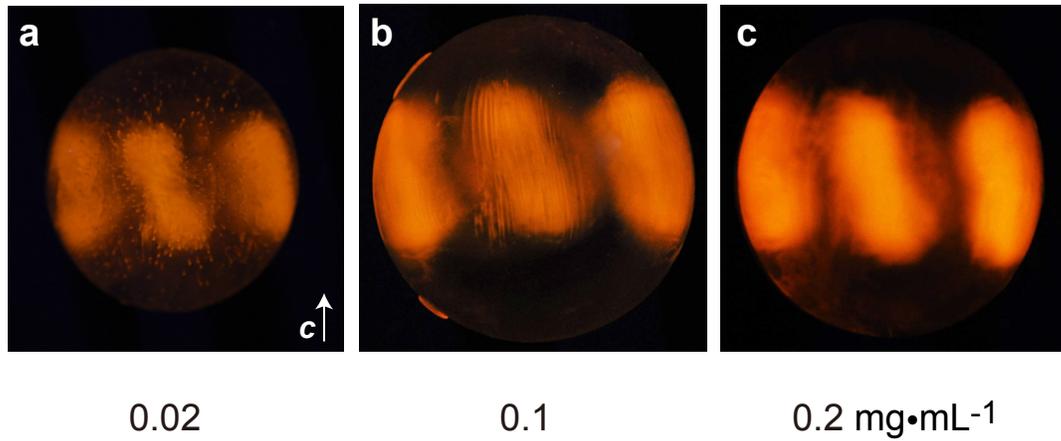
Supplementary Figure S1. Preparation of bpAFP sample. **(a)** Reversed-phase HPLC chromatogram (TSKgel ODS-80Ts column, TOSOH, Tokyo, Japan) of native isoform mixture of barfin plaice AFP. The bpAFP isoform was eluted by a linear gradient of 0-100% acetonitrile in 0.1% trifluoroacetic acid (dashed line). **(b)** Electrophoretogram of the recombinant bpAFP (rbpAFP) separated by 15 % Tricine SDS-PAGE with molecular weight markers indicated on the left.

Supplementary Figure S2



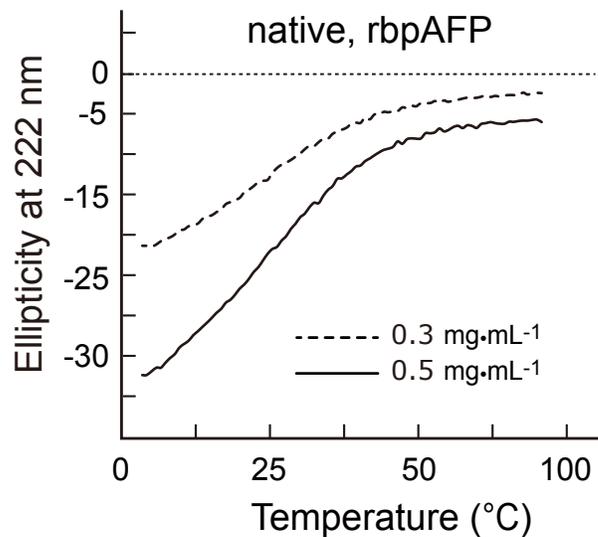
Supplementary Figure S2. Ice-binding affinity of rbpAFP. (a) The fluorescence- based ice plane affinity (FIPA) of rhodamine®-tagged rbpAFP at a concentration of 0.02 mg·mL⁻¹ (i), and its interpreted illustration (ii). (b) Change of the FIPA pattern with increasing the rbpAFP concentration. Upper (i-iv) and lower panels (v-viii) show top and side views of an ice hemisphere, respectively. The direction of the *c*-axis is shown by the white circle (upper) and arrow (lower).

Supplementary Figure S3



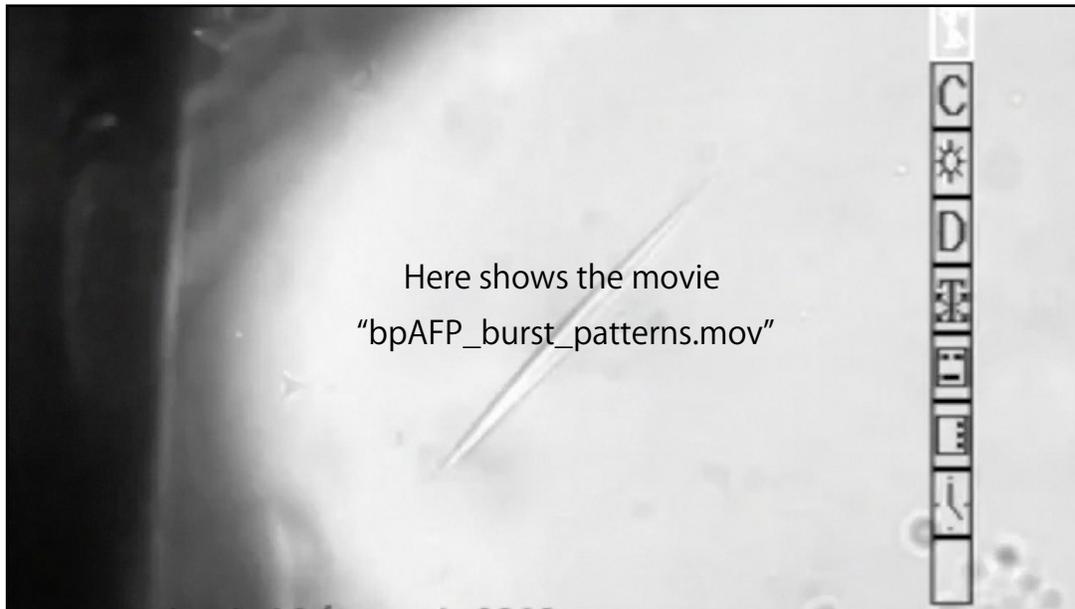
Supplementary Figure S3. Fluorescence-based ice plane affinity (FIPA) analysis of recombinant nfeAFP6 isoform of type III AFP from Notched-fin eelpout, *Zoarces elongatus* Kner. Unlike bpAFP, nfeAFP6 does not bind to the whole ice hemisphere surface even at a concentration of 0.2 mg/mL. The direction of the *c*-axis is shown by the white arrow.

Supplementary Figure S4



Supplementary Figure S4. Change of the mean residue ellipticity at 222 nm of native- and recombinant bpAFP as a function of temperature. The same profile was obtained for these peptides at the concentrations of 0.01, 0.02, 0.03, 0.06, 0.08, 0.1, 0.2, 0.3, 0.4, and 0.5 mg·mL⁻¹, suggesting that bpAFP undergoes no critical transition between monomer and oligomer.

Supplementary Movie 1



Supplementary Movie 1. Movie files showing two different patterns of bursting ice crystal growth from an ice bipyramid in the presence of recombinant bpAFP (rbpAFP). The first 6-second movie shows a crystal bursting along with the *c*-axis of the ice bipyramid in 5 m/mL rbpAFP, and in the last 6-second movie the burst pattern in 150 mg/mL rbpAFP is normal to the *c*-axis. Figure 2 in the main text shows four snapshots from each pattern.