Electronic Supplementary Material (ESI) for RSC Advances.

Experimental Correlation between Thermal Hysteresis Activity and the Distance between Antifreeze Proteins on an Ice Surface

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Supporting information

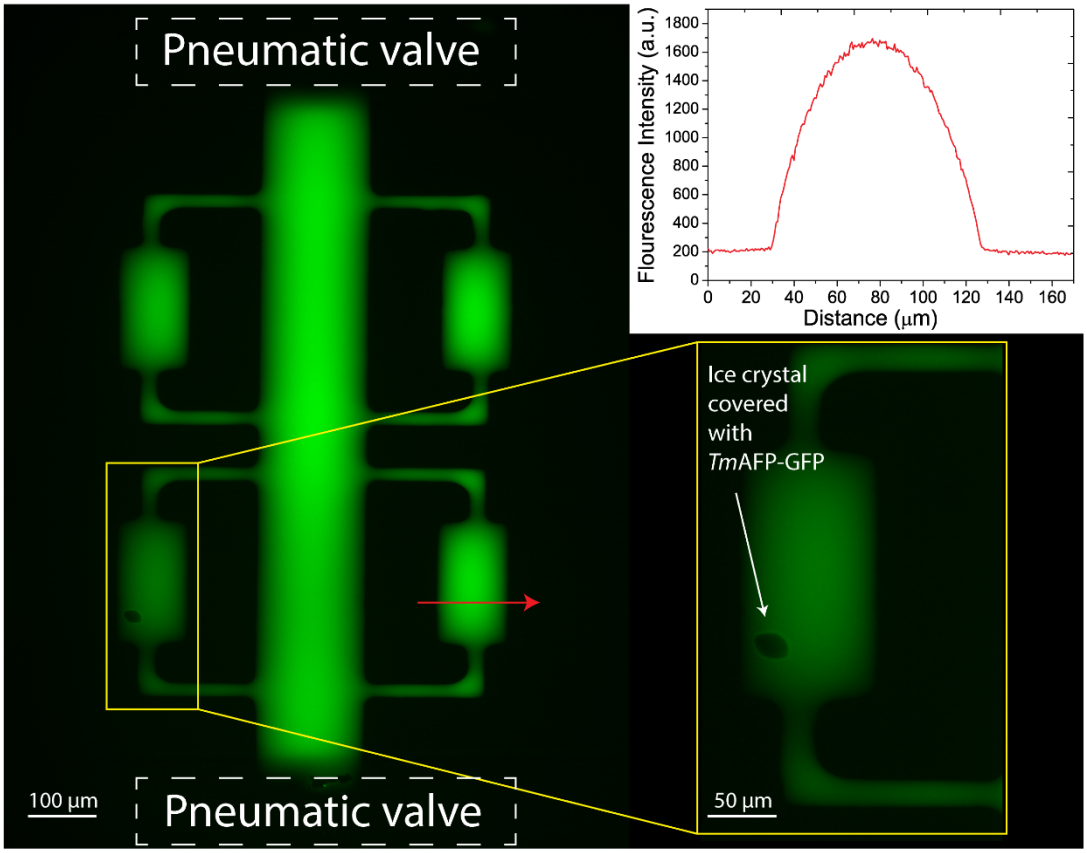


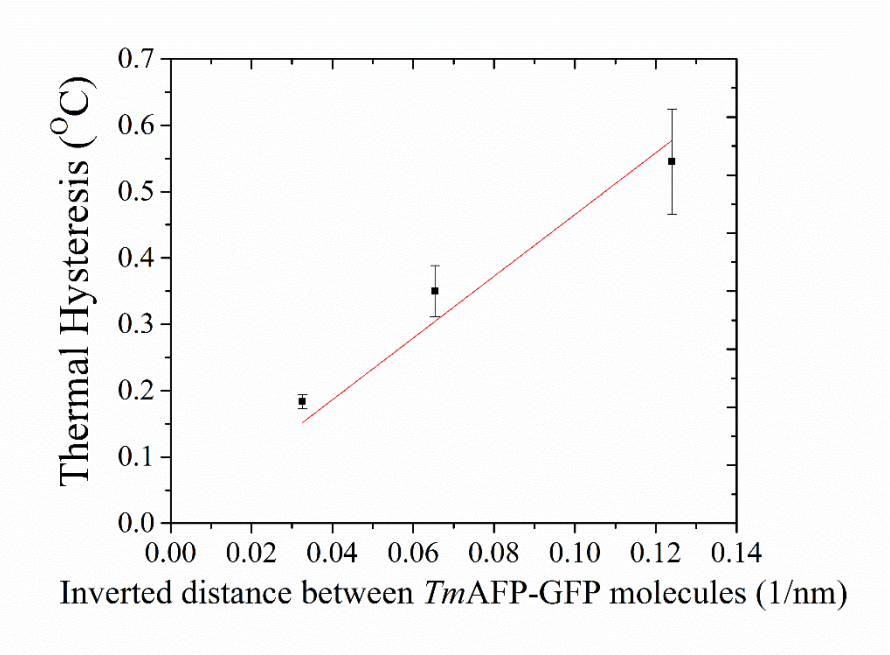
Figure S1 – Structure of the microfluidic channel. The microfluidic channels include two layers – flow and control. The flow layer contains the liquid and the ice crystals, and the control layer includes immersion oil and functions as pneumatic valve system. The flow layer has to be rounded (see intensity graph above) to ensure that after the valve is closed, liquid cannot pass through the valve. Adopted with permission from Drori *et al.* 2014[11].

S2 – *Tm*AFP-GFP surface concentration measurements data. Each row in the table is one crystal that was exposed to the corresponding *Tm*AFP-GFP concentration, during the stated exposure time. Then the solution was exchanged and TH was measured.

Solution concentration before exchange (μM)	Exposure time (min)	Solution concentration after exchange (μM)	TH	distance (nm)
31.4	184	2.6	0.74	7.6
20.8	12	1.8	0.52	7.9
10.0	5	0.9	0.5	8.5
9.8	12	1.5	0.19	25.5
7.6	10	2.2	0.42	8.3
7.4	76	0.4	0.42	12.2
6.2	23	1.0	0.2	33.2
5.4	73	0.8	0.38	13.8
4.8	62	2.0	0.24	17.4
0.5	20	0.3	0.36	20.0
0.4	4	0.3	0.16	35.2

S3 –AFPIII-GFP surface concentration measurements data. Each row in the table is one crystal that was exposed to the corresponding AFPIII-GFP concentration, the exposure time for all of these crystals was 3-5 min.

Solution concentration before excahnge (μM)	Solution concentration after exchange (μM)	TH (^{o}C)	distance (nm)
19.8	0.1	0.10	17.5
8.8	0.5	0.04	13.6
5.7	0.1	0.04	24.7
5.7	0.2	0.11	16.8
4.2	0.1	0.09	15.9
3.3	1.5	0.15	8.7
2.6	0.6	0.11	14.8
1.9	0.4	0.08	14.8
1.5	0.1	0.09	17.5
1.3	0.2	0.04	15.9
1.2	0.3	0.04	16.8



S4 – Average of the reciprocal distance between *Tm*AFP-GFP molecules (figure 3). The data points were binned to clusters containing 3 or 4 data points in each cluster. This curve was fitted using the same fitting formula as shown in figure 3 ($TH = \frac{4.6\,nm\,K}{d}$). The R-squared value of this curve is 0.94.