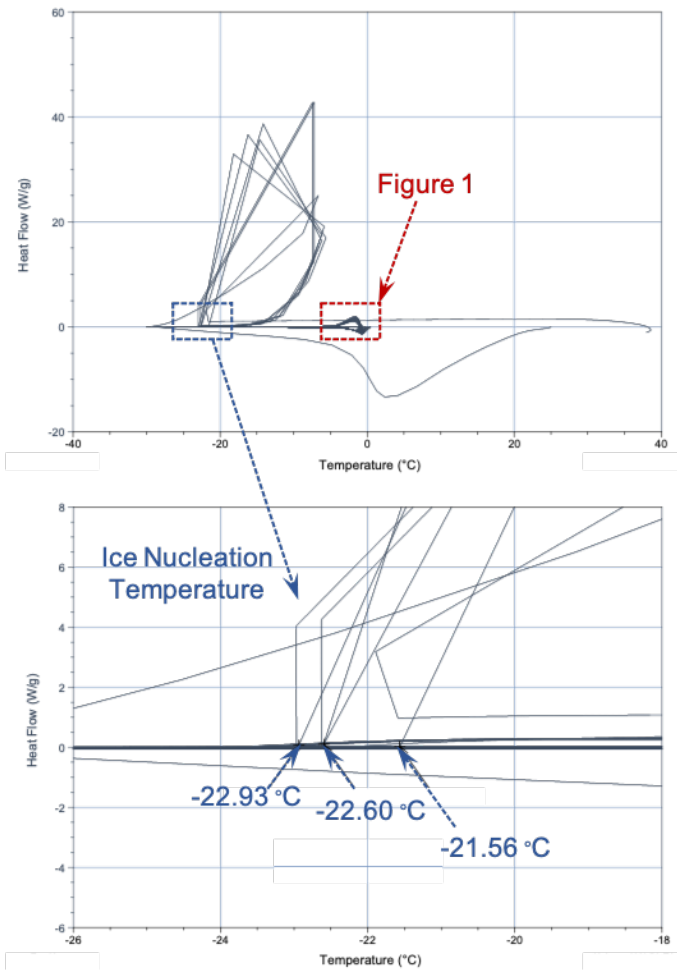


a



b

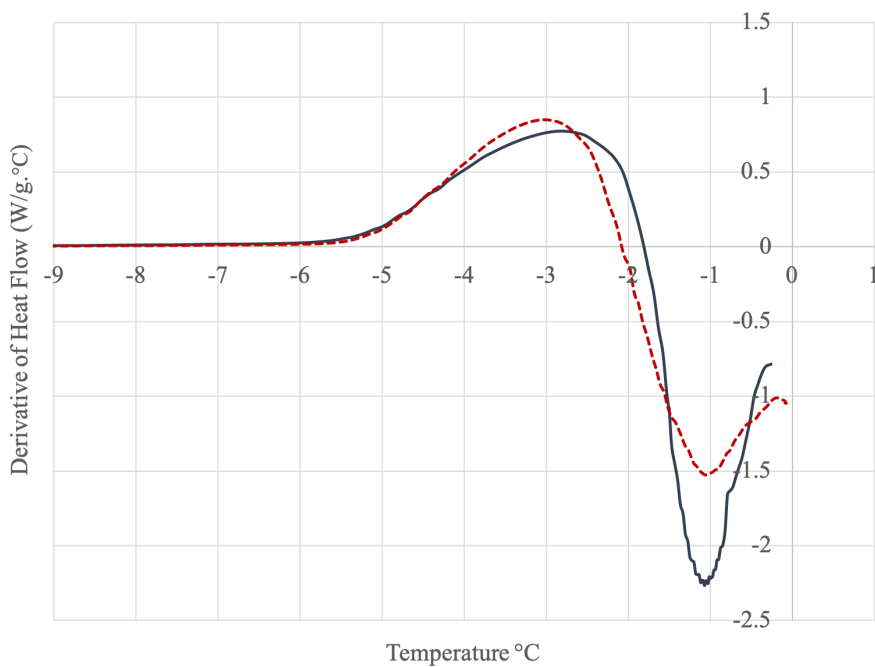


Fig. S1. Ice nucleation temperature for 180 μ M solution of Trx-ApAFP752 in buffer (50 mM K₂HPO₄, 20 mM NaCl, pH 8) analyzed via differential scanning calorimetry.

a) The top panel is the full temperature range showing all cycles, with indication of what portion of the figure was used for Figure 1 in the text. The bottom panel is a focused view for later heating cycles which show 3 repetitions of cooling cycles and their respective nucleation temperatures observed as large exotherms. b) THA activity is retained after lyophilization. Differential scanning calorimetry (DSC) was used to assay thermal hysteresis for the antifreeze protein (180 μ M solution of AFP in buffer: 50 mM K₂HPO₄, 20 mM NaCl, pH 8). Here, two samples are contrasted of the Trx-ApAFP752 protein (pre-lyophilization in solid dark blue, after lyophilization in dashed red) highlighting only the specific cooling cycle used to determine thermal hysteresis for those samples. In contrast to Figure 1 in the main text, here we plot the derivative of the heat flow for that cycle which allows us to more easily observe the rapid change in slope at the onset of freezing near the first minimum just under 0 °C. This depression in freezing point (i.e., THA) was observed to be the same for the protein both before and after lyophilization.

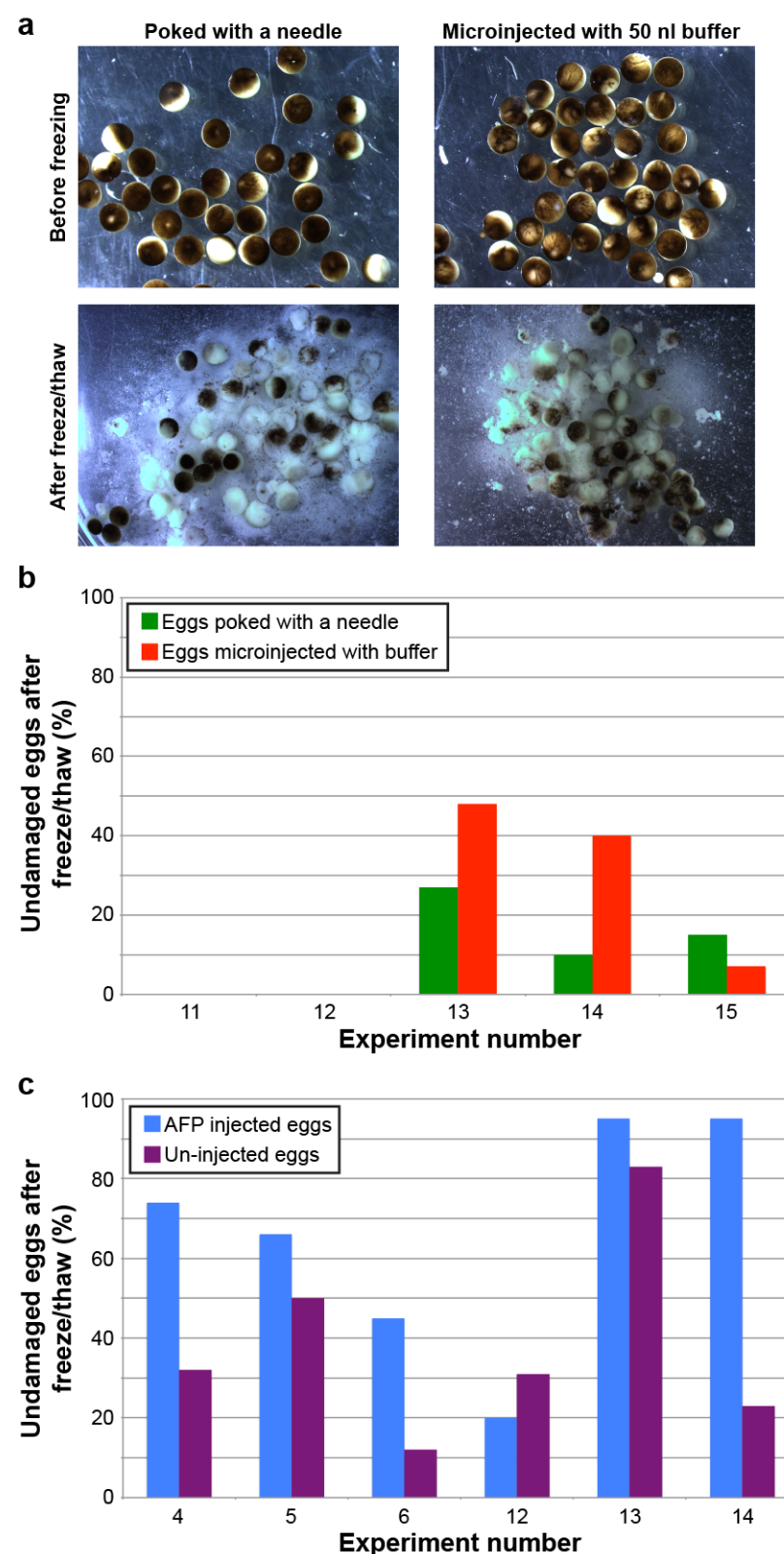


Fig. S2. Puncturing eggs renders them susceptible to freeze/thaw damage, and un-injected eggs exhibit variable sensitivity to freezing. a) Eggs were punctured with a microinjection needle or microinjected with 50 nL of buffer and subjected to freeze/thaw, as described in Figure 3. Representative images are shown. b) Quantification of data from a). For each approach, five independent experiments were performed using 10-40 eggs per experiment. The average \pm SD for percentage of undamaged eggs was $10 \pm 11\%$ for poked eggs and $19 \pm 23\%$ for buffer injected eggs, and the difference between the two conditions was not statistically significant by Student's t-test. c) Experiments were performed as in Figure 3 with eggs that were either un-injected or microinjected with $5 \mu\text{M}$ AFP. Experimental conditions are summarized in Table S1.

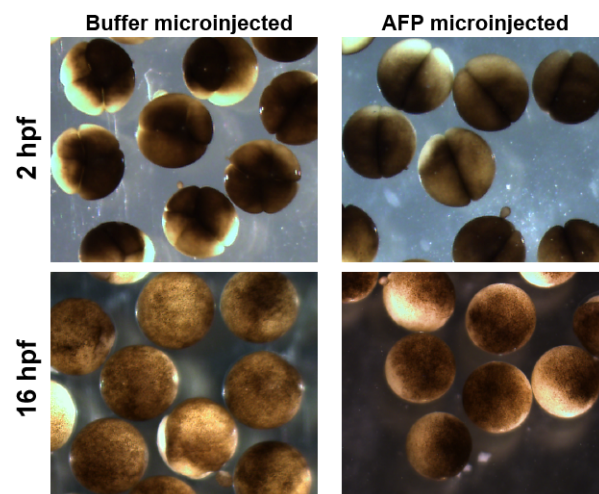


Fig. S3. AFP is not toxic for early *X. laevis* development. One-cell *X. laevis* embryos were microinjected with 5 μ M AFP or buffer as described in Figure 3. Embryos were not subjected to freeze/thaw, allowed to develop at room temperature, and imaged at different times, indicated in hours post fertilization (hpf). Two independent experiments were performed using different batches of embryos. Table S3 summarizes the number of embryos that were injected at the one-cell stage, the number of embryos that reached gastrulation (16 hpf), and microinjection conditions for each experiment.

Table S1. Compiled microinjection data for eggs. All data from egg microinjections are shown.

Exp. #	Microinjection	Freeze/thaw condition	AFP used	Total # of eggs	% undamaged eggs
1	5 μ M Trx-ApAFP ^I	A	Batch 1: fresh	50	96%
	Buffer ^{II}	A		30	13%
2	5 μ M Trx-ApAFP ^I	A	Batch 1: fresh	61	75%
	Buffer ^{II}	A		64	50%
3	5 μ M Trx-ApAFP ^I	A	Batch 1: fresh	93	67%
	Buffer ^{II}	A		105	41%
4	5 μ M Trx-ApAFP ^I	B	Batch 2: fresh	39	74%
	5 μ M heat-inactivated Trx-ApAFP ^{III}	B	Batch 2: heat-inactivated	22	9%
	Not microinjected	B		19	32%
5	5 μ M Trx-ApAFP ^I	A	Batch 2: fresh	29	66%
	5 μ M heat-inactivated Trx-ApAFP ^{III}	A	Batch 2: heat-inactivated	15	7%
	Not microinjected	A		12	50%
6	5 μ M Trx-ApAFP ^I	A	Batch 2: fresh	22	45%
	5 μ M heat-inactivated Trx-ApAFP ^{III}	A	Batch 2: heat-inactivated	21	5%
	Not microinjected	A		17	12%
7	5 μ M Trx-ApAFP ^I	A	Batch 2: 3 weeks at 4°C	60	47%
	5 μ M heat-inactivated Trx-ApAFP ^{III}	A	Batch 2: heat-inactivated	63	32%
8	5 μ M Trx-ApAFP ^I	C	Batch 3: fresh	94	63%
	5 μ M heat-inactivated Trx-ApAFP ^{III}	C	Batch 3: heat-inactivated	103	35%
9	5 μ M Trx-ApAFP ^I	C	Batch 3: 2 weeks at 4°C	15	73%
	5 μ M heat-inactivated Trx-ApAFP ^{III}	C	Batch 3: heat-inactivated	15	13%
	5 μ M lyophilized Trx-ApAFP ^I	C	Batch 3: lyophilized	15	73%
	2 μ M lyophilized Trx-ApAFP ^{IV}	C	Batch 3: lyophilized	15	13%
10	5 μ M Trx-ApAFP ^I	C	Batch 3: 2 weeks at 4°C	33	58%
	5 μ M heat-inactivated Trx-ApAFP ^{III}	C	Batch 3: heat-inactivated	39	18%
	5 μ M lyophilized Trx-ApAFP ^I	C	Batch 3: lyophilized	39	41%

	2 μ M lyophilized Trx-ApAFP ^{IV}	C	Batch 3: lyophilized	36	19%
11	5 μ M Trx-ApAFP ^I	A	Batch 4: fresh	9	100%
	Buffer ^{II}	A		10	0%
	Not microinjected	A		10	20%
	Poked with a microinjection needle	A		10	0%
12	5 μ M Trx-ApAFP ^I	A	Batch 4: fresh	15	20%
	Buffer ^{II}	A		16	0%
	Not microinjected	A		13	31%
	1 μ M Trx-ApAFP ^V	A	Batch 4: fresh	15	0%
	Poked with a microinjection needle	A		16	0%
13	5 μ M lyophilized Trx-ApAFP ^I	A	Batch 3: lyophilized (aged)	40	95%
	Buffer ^{II}	A		40	48%
	Not microinjected	A		40	83%
	1 μ M lyophilized Trx-ApAFP ^V	A	Batch 3: lyophilized (aged)	40	38%
	Poked with a microinjection needle	A		40	27%
14	5 μ M lyophilized Trx-ApAFP ^I	A	Batch 3: lyophilized (aged)	40	95%
	Buffer ^{II}	A		40	40%
	Not microinjected	A		40	23%
	1 μ M lyophilized Trx-ApAFP ^V	A	Batch 3: lyophilized (aged)	40	45%
	Poked with a microinjection needle	A		40	10%
15	Buffer ^{II}	A		41	7%
	Poked with a microinjection needle	A		41	15%

Buffer = 20 mM Tris, 20 mM NaCl, pH 7.5

Each experiment was conducted with a different batch of unfertilized *X. laevis* eggs

A: Eggs were equilibrated in 0.5x Ringer + 6% Ficoll prior to microinjection. After microinjection, eggs were incubated in the same buffer for 1 hour at room temperature, moved to 5 ml 0.5x Ringer without Ficoll, and placed at -20°C for 15 minutes until the buffer had frozen. Eggs were then thawed at room temperature for 45 minutes and imaged. Cooling at -1.0 °C/min; thawing at +0.3 °C/min.

B: Eggs were equilibrated in 0.5x Ringer + 6% Ficoll prior to microinjection. After microinjection, eggs were incubated in the same buffer for 45 minutes at room temperature and moved to 5 ml 0.5x Ringer without Ficoll. The dish of eggs in buffer was then placed in a surrounding iso-propanol bath at -20°C overnight. The iso-propanol bath ensures the eggs freeze slowly. Note that the eggs themselves were not placed directly in iso-propanol. As such, the buffer and eggs ultimately reached -20 °C and froze. After overnight at -20 °C, eggs were then thawed at room temperature for 45 minutes and imaged. Cooling at -0.7 °C/min; thawing at +0.3 °C/min.

C: Eggs were equilibrated in 0.5x Ringer + 6% Ficoll prior to microinjection. After microinjection, eggs were incubated in the same buffer for 45 minutes at room temperature, moved to 5 ml 0.5x Ringer/10% DMSO/20% FBS, placed on ice for 30 minutes, and placed in an iso-propanol bath at -20°C overnight (as described for condition B). Eggs were then thawed at room temperature for 45 minutes and imaged. Cooling at -0.8 °C/min; thawing at +0.2 °C/min.

^I Microinjected 50 nl of 105-117 μ M Trx-ApAFP stock

^{II} Microinjected 50 nl of buffer

^{III} Microinjected 50 nl of 105-117 μ M Trx-ApAFP stock that had first been heat-inactivated at 55°C for 15 minutes

^{IV} Microinjected 50 nl of 50 μ M Trx-ApAFP stock

^V Microinjected 10 nl of 105 μ M Trx-ApAFP stock

Aged: Lyophilized and stored at room temperature for ~17 months

Table S2. Compiled microinjection data for embryos. All data from embryo microinjections are shown.

Exp. #	Microinjection	AFP used	Total # of embryos	% undamaged embryos	% embryos developed
1	5 μ M Trx-ApAFP ^I	Batch 1: fresh	20	80%	ND
	Buffer ^{II}		20	30%	ND
2	5 μ M Trx-ApAFP ^I	Batch 2: fresh	11	88%	36% ^G
	5 μ M heat-inactivated Trx-ApAFP ^{III}	Batch 2: heat-inactivated	11	18%	0% ^G
	Not microinjected		10	10%	0% ^G
3	5 μ M Trx-ApAFP ^I	Batch 2: fresh	24	63%	30% ^{St7}
	5 μ M heat-inactivated Trx-ApAFP ^{III}	Batch 2: heat-inactivated	20	30%	5% ^{St7}
4	5 μ M Trx-ApAFP ^I	Batch 3: fresh	20	5%	ND
	5 μ M heat-inactivated Trx-ApAFP ^{III}	Batch 3: heat-inactivated	20	20%	ND
	Not microinjected		20	35%	ND
5	5 μ M Trx-ApAFP ^I	Batch 3: fresh	40	55%	ND
	5 μ M heat-inactivated Trx-ApAFP ^{III}	Batch 3: heat-inactivated	40	8%	ND
	Not microinjected		40	88%	ND
6	5 μ M Trx-ApAFP ^I	Batch 4: fresh	20	25%	ND
	Buffer ^{II}		20	30%	ND
	Not microinjected		20	40%	ND
7	5 μ M lyophilized Trx-ApAFP ^I	Batch 4: lyophilized	20	10%	ND
	Buffer ^{II}		20	10%	ND
	Not microinjected		20	30%	ND
	1 μ M lyophilized Trx-ApAFP ^{IV}	Batch 4: lyophilized	20	15%	ND
8	GFP-AFP mRNA ^V	mRNA	15	40%	ND
	AFP mRNA ^V	mRNA	15	47%	ND
	Water ^{VI}		15	7%	ND
	Not microinjected		15	0%	ND
9	GFP-AFP mRNA ^V	mRNA	15	13%	ND
	AFP mRNA ^V	mRNA	15	20%	ND
	Water ^{VI}		15	20%	ND
	Not microinjected		15	33%	ND

Buffer = 20 mM Tris, 20 mM NaCl, pH 7.5

Each experiment was conducted with a different batch of *X. laevis* embryos

Freeze/thaw conditions for AFP recombinant protein microinjection experiments #1-7: One-cell stage embryos were equilibrated in 0.5x Ringer + 6% Ficoll prior to microinjection. After microinjection, embryos were incubated in the same buffer at room temperature until they reached stage 4-5. Embryos were then moved to 5 ml 0.5x Ringer without Ficoll, and placed at -20°C for 15 minutes until the buffer had frozen. Embryos were then thawed at room temperature for 45 minutes and imaged.

Freeze/thaw conditions for AFP mRNA microinjection experiments #8-9: One-cell stage embryos were equilibrated in 0.5x Ringer + 6% Ficoll prior to microinjection. After microinjection, embryos were incubated at room temperature until they reached stage 8-9. Embryos were placed in 5 ml 0.5x Ringer without Ficoll at -20°C for 15 minutes until the buffer had frozen. Embryos were then thawed at room temperature for 45 minutes and imaged.

^I Microinjected 50 nl of 105-117 μ M Trx-ApAFP stock

^{II} Microinjected 50 nl of buffer

^{III} Microinjected 50 nl of 105-117 μ M Trx-ApAFP stock that had first been heat-inactivated at 55°C for 15 minutes

^{IV} Microinjected 10 nl of 105 μ M Trx-ApAFP stock

^V Microinjected 500 pg mRNA

^{VI} Microinjected 10 nl of water

ND Not determined

^G Reached gastrula stage

^{St7} Reached stage 7

Table S3. Compiled data for embryos that were microinjected, not subjected to freeze/thaw, and allowed to develop at room temperature. All data from embryo microinjections are shown.

Exp. #	Microinjection	AFP used	Total # of embryos injected at 1-cell stage	Total # of viable embryos at 16 hpf
1	5 µM Trx-ApAFP ^I	Batch 1: fresh	70	55 ^G
	Buffer ^{II}		68	57 ^G
2	5 µM Trx-ApAFP ^I	Batch 2: fresh	20	16 ^G
	Buffer ^{II}		20	16 ^G

One-cell stage embryos were equilibrated in 0.5x Ringer + 6% Ficoll prior to microinjection. After microinjection, embryos were incubated in the same buffer at room temperature until they reached stage 4-5. Embryos were then moved to 0.5x Ringer without Ficoll and incubated at room temperature.

Buffer = 20 mM Tris, 20 mM NaCl, pH 7.5
Each experiment was conducted with a different batch of *X. laevis* embryos

^I Microinjected 50 nl of 105-117 µM Trx-ApAFP stock
^{II} Microinjected 50 nl of buffer

^G Reached gastrula stage