Supplementary Material to the manuscript **"Thermal inactivation of a cold-active esterase PMGL3 isolated from the permafrost metagenomic library"** by M.V. Kryukova et al.

Table S1. Content of the secondary structure elements in PMGL3 and mutant variants obtained by CD spectroscopy at different temperatures.

Protein	**Temperature, °C**	**α-helix,** %	**ß-sheet,** %	**unregular,** %
PMGL3	20	31.3	16.6	52.1
	30	30.6	18.1	51.3
	40	27.3	20.5	52.1
	50	15.6	27.9	56.5
C49V	20	32.8	17.8	49.5
	30	31.0	17.5	51.6
	40	27.8	19.2	53.0
	50	17.6	26.9	55.5
C207F	20	29.6	22.7	47.7
	30	28.2	23.7	48.0
	40	27.4	22.7	49.9
	50	16.2	31.4	52.4

Figure S1. Location of cysteine residues and residues belonging to the catalytic triad in the PMGL3 molecule. Ribbon presentations are colored according to the predicted secondary structure of the protein; green and blue elements indicate α-helices and β-sheets, respectively. A structure model of PMGL3 was generated using SWISS-MODEL server based on PDB entry 5GMR as a template and drawn using Swiss-PdbViewer.

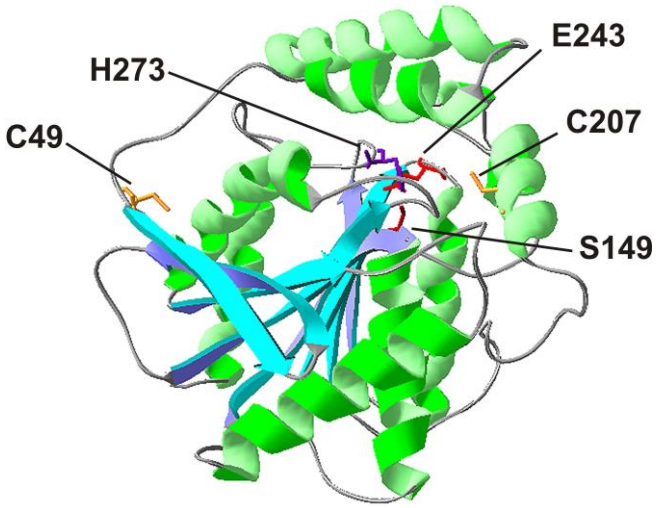


Figure S2. Characteristic fluorescence decay kinetics of bis-ANS in the buffer and in protein solutions of PMGL3 and mutant variants at 25°C.

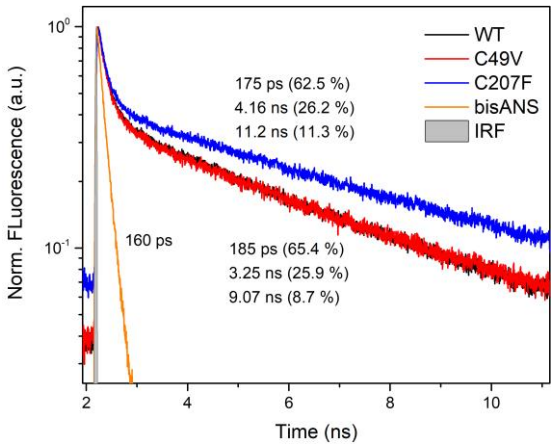
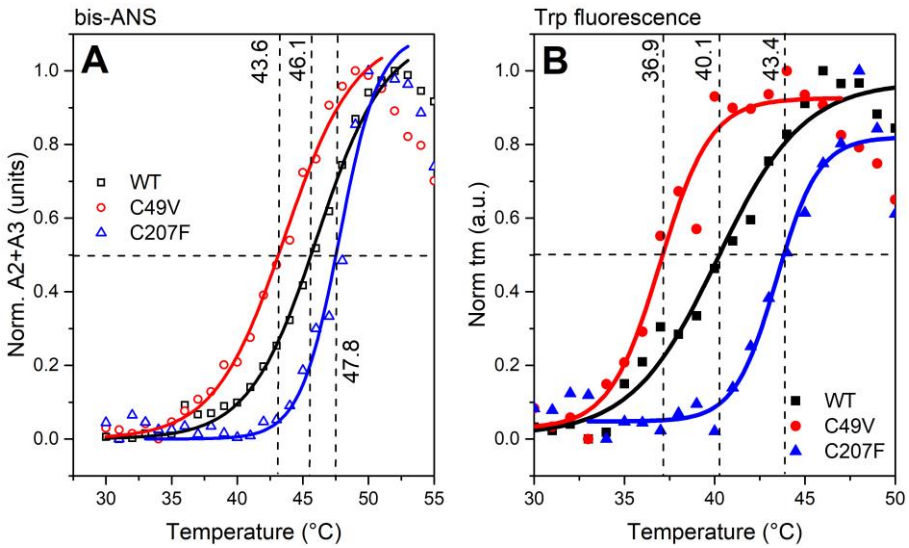


Figure S3. Melting of PMGL3 and mutant variants as revealed by bis-ANS fluorescence (**A**) and intrinsic Trp fluorescence (**B**).


bis-ANS
A
43.6
46.1
47.8
WT
C49V
C207F
Norm. A2+A3 (units)
Temperature (°C)
Trp fluorescence
B
36.9
40.1
43.4
WT
C49V
C207F
Norm tm (a.u.)
Temperature (°C)