Supplementary Information

Thermodynamic instability of viral proteins is a pathogen-associated molecular pattern targeted by human defensins

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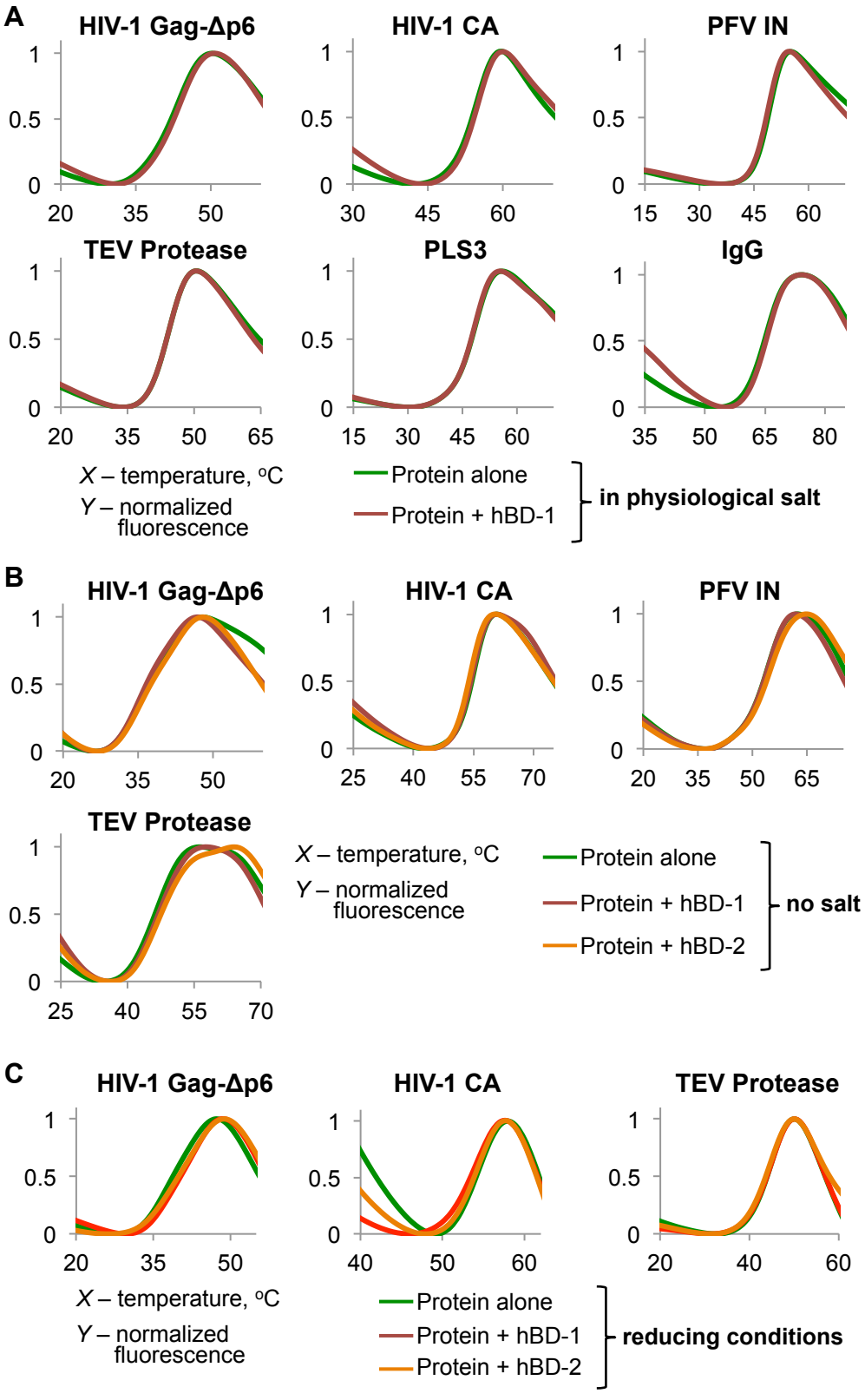
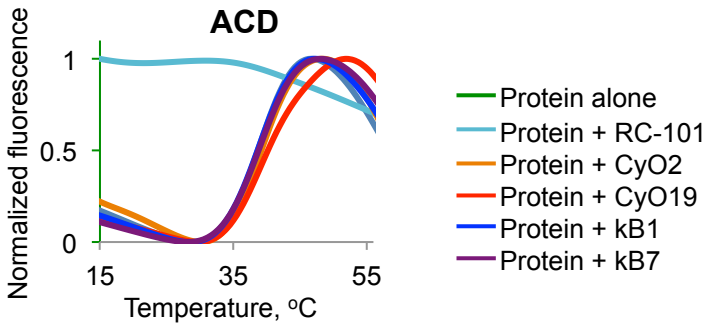
Supplementary Figure S1



Supplementary Figure S1. β-Defensins do not cause unfolding of viral proteins. (**A**) Thermal denaturation profiles of viral proteins (HIV-1 Gag-Δp6, HIV-1 CA, PFV IN, and TEV protease) and mammalian proteins (PLS3 and IgG) were tested in the absence (green lines) and presence of 3-molar excess of β-defensin hBD-1 (red lines) at physiological salt concentration. (**B**) In the absence of salt (20 mM HEPES, pH 7.5), β-defensins hBD-1 and hBD-2 (orange lines) do not affect melting temperatures of the tested proteins (HIV-1 Gag-Δp6, HIV-1 CA, PFV IN, and TEV protease). (**C**) Under reducing conditions (10 mM TCEP), β-defensins hBD-1 and hBD-2 do not affect melting of the tested proteins (HIV-1 Gag-Δp6, HIV-1 CA, and TEV protease).

Supplementary Figure S2



Supplementary Fig. S2. Cyclotides do not cause unfolding of a thermolabile bacterial protein toxin. DSF thermal denaturation profile of Actin Crosslinking Domain (ACD) toxin form *V. cholerae* was shifted toward lower temperature in the presence of 5-molar excess of RC-101, but not in the presence of any of the tested cyclotides (in 5-molar excess to the toxin).

Supplementary Table S1. Anti-microbial peptides (AMP) used in this study

AMP name	AMP sequence / (total number of residues)	Arg/10[1]	Lys/10[2]	His/10[3]	"+"/10[4]	"-"/10[5]	GRAVY[6]	Monomer MW, kDa[7]
HNP-1	ACYCRIPACIAGERRYGTCIYQ GRLWAFCC (30)	1.33	0	0	1.33	0.33	0.300	3.45
HD-5	ATCYCRTGRCATRESLSGVCEI SGRLYRLCCR (32)	1.88	0	0	1.88	0.63	-0.113	3.59
hBD-1	DHYNCVSSGGQCLYSACPIFTK IQGTCYRGKAKCCK (36)	0.28	1.11	0.28	1.39	0.28	-0.272	3.93
hBD-2	GIGDPVTCLKSGAICHPVFCPR RYKQIGTCGLPGTKCCKKP (41)	0.49	1.21	0.24	1.71	0.24	-0.102	4.33
RC-101	GICRCICGKGICRCICGR (18; cyclic)	1.67	0.56	0	2.22	0	0.778	1.91
cyO2	GIPCGESCVWIPCISSAIGCSCK SKVCYRN (30; cyclic)	0.33	0.67	0	1.00	0.33	0.443	3.16
cyO19	GTLPCGESCVWIPCISSVVGCS CKSKVCYKD (31; cyclic)	0	0.97	0	0.97	0.65	0.471	3.25
kB1	GLPVCGETCVGGTCNTPGCTC SWPVCTRN (29; cyclic)	0.34	0	0	0.34	0.34	0.152	2.92
kB7	GLPVCGETCTLGTCYTQGCTC SWPICKRN (29; cyclic)	0.34	0.34	0	0.68	0.34	0.038	3.10

Acidic residues are shown in red, basic residues are in blue, hydrophobic uncharged residues are in green, cysteines are in bold.

Parameters calculated using ExPASy ProtParam tool:

[1] – Number of arginines per 10 residues
[2] – Number of lysines per 10 residues
[3] – Number of histidines per 10 residues
[4] – Number of positively charged residues per 10 residues
[5] – Number of negatively charged residues per 10 residues
[6] – Grand average of hydropathicity (GRAVY)
[7] – Molecular weight (MW), kDa