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Supplementary Material

Mechanisms of a Small Membrane-Active Antimicrobial Peptide from *Hyla punctata*

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Table S1

Peptide	Source	Sequence	Similarity to HSP1 [%]	Total Charge	$\Delta G_{\mathrm{Interface}}$ [kcal/mol]
HSP1	*Hyla punctata* *South America*	GIL**D**AI**K**AIA**K**AAG	-	+1	2.32
Hylain 1	*Hyla simplex* *China*	GIL**D**AI**K**AFANALG	79	0	0.20
Hylain 2	*Hyla simplex* *China*	GIL**D**PI**K**AFA**K**AAG	86	+1	1.78
Aurein 1.2	*Litoria raniformis* *Australia*	GLF**D**II**KK**IA**E**SF	60	0	1.52

Table S1. Summary of the peptide sequences of HSP1 and their analogues. Assume Ile and Leu have similar properties and are identical in the similarity analysis. The similiarity percentage was calculated by Align Sequences Protein BLAST software from National Center for Biotechnology Information at U.S. National Library of Medicine <https://blast.ncbi.nlm.nih.gov/>. $\Delta G_{\mathrm{Interface}}$ shows the free energy from water to POPC interface, and was estimated using Wimley-White Scale in MPEx software <http://blanco.biomol.uci.edu/mpex/>.

Table S2

Peptide	Ultrapure Water	1x DPBS (pH 7.1±0.1)	DMSO
HSP1	5 mg/mL	< 0.1 mg/mL	5 mg/mL

Table S2. Peptide solubility test of HSP1. Solubility test was provided by the distributor, GenScript, Inc. DMSO: dimethyl sulfoxide (analytical grade). DPBS: Dulbecco's phosphate buffered saline, containing potassium chloride (KCl), potassium phosphate monobasic (KH_2PO_4), sodium chloride (NaCl) and sodium phosphate diabsic (Na_2HPO_4-$7H_2O$).

Figure S1

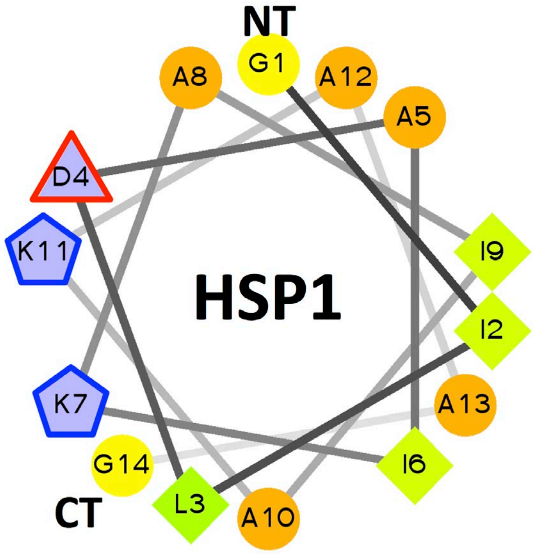

Figure S1. Helical wheel projection of HSP1 and their analogues showing their hydrophobicity in color: the most hydrophobic amino acid is shown as green, and the amount of green is decreasing proportionally to the hydrophobicity. Zero hydrophobicity presents as yellow. Polar (uncharged) residues are coded red, and the amount of red is decreasing proportionally to the hydrophilicity. The potentially charged residues are light blue, and the negatively charged is triangle with red border and the positively charged is pentagon with blue border.

Figure S2

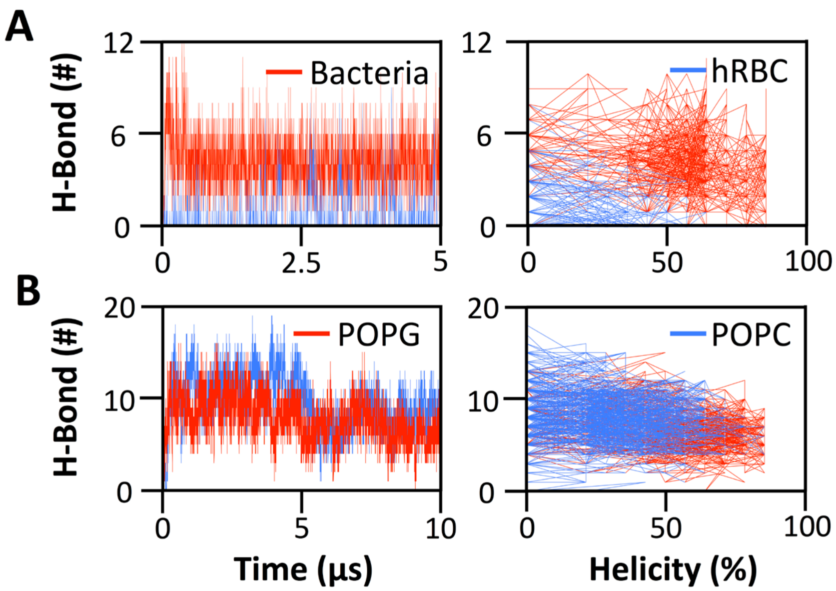

Figure S2. Illustration of the fast folding and adsorption process from the initial water-solvated unfolded state (W). Hydrogen bond analysis of protein and lipids **(Left panel)** and corresponding of the hydrogen bond of protein and lipids versus the peptide helicity **(Right panel)** of HSP1 folding with **(A)** bacterial and hRBC model membranes using CHARMM forcefield, **(B)** POPG and POPC bilayers using OPLS-ua forcefield.