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Supplementary Info

Surfactin-inspired arginine- and lysine-rich peptides inhibit human insulin aggregation and prevent amyloid-induced cytotoxicity

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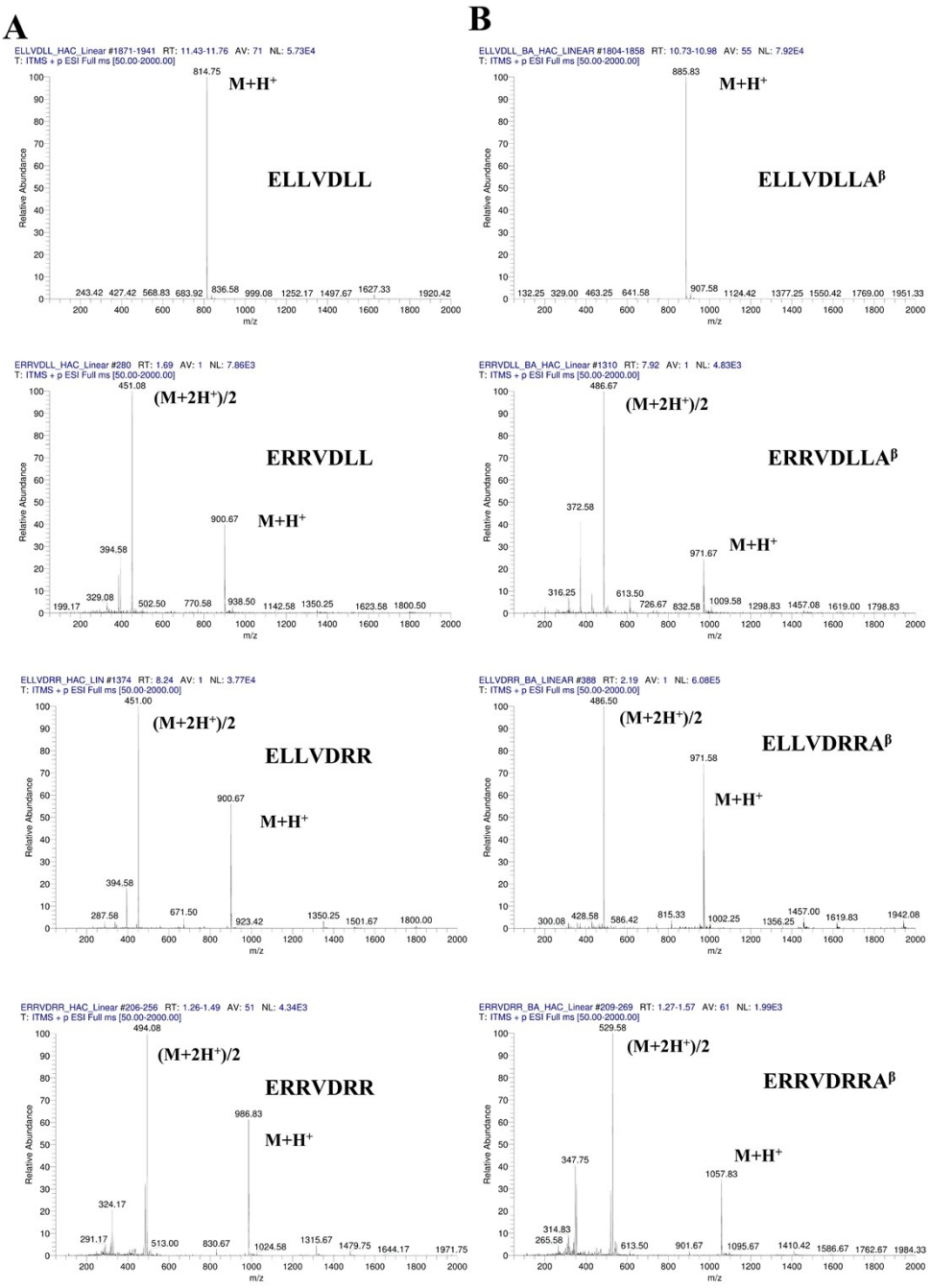
A
B
ELLVDLL_HAC_Linear #1871-1941 RT: 11.43-11.76 AV: 71 NL: 5.73E4
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M+H+
ELLVDLL
ELLVDLL_BA_HAC_LINEAR #1804-1858 RT: 10.73-10.98 AV: 55 NL: 7.92E4
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M+H+
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451.08
(M+2H+)/2
ERRVDLL
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M+H+
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ERRVDLLAβ
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971.67
M+H+
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(M+2H+)/2
ELLVDRR
900.67
M+H+
ELLVDRR_BA_LINEAR #388 RT: 2.19 AV: 1 NL: 6.08E5
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486.50
(M+2H+)/2
ELLVDRRAβ
971.58
M+H+
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(M+2H+)/2
ERRVDRR
986.83
M+H+
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ERRVDRRAβ
347.75
1057.83
M+H+
Relative Abundance
m/z

I

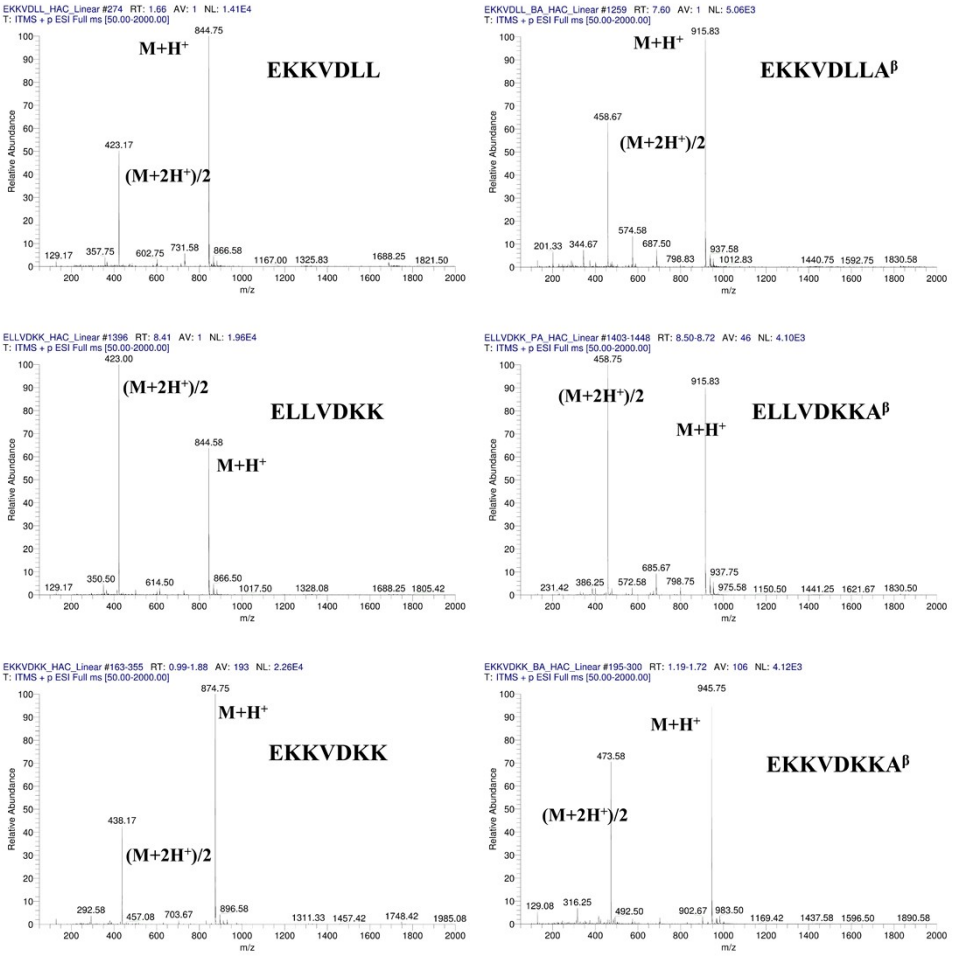
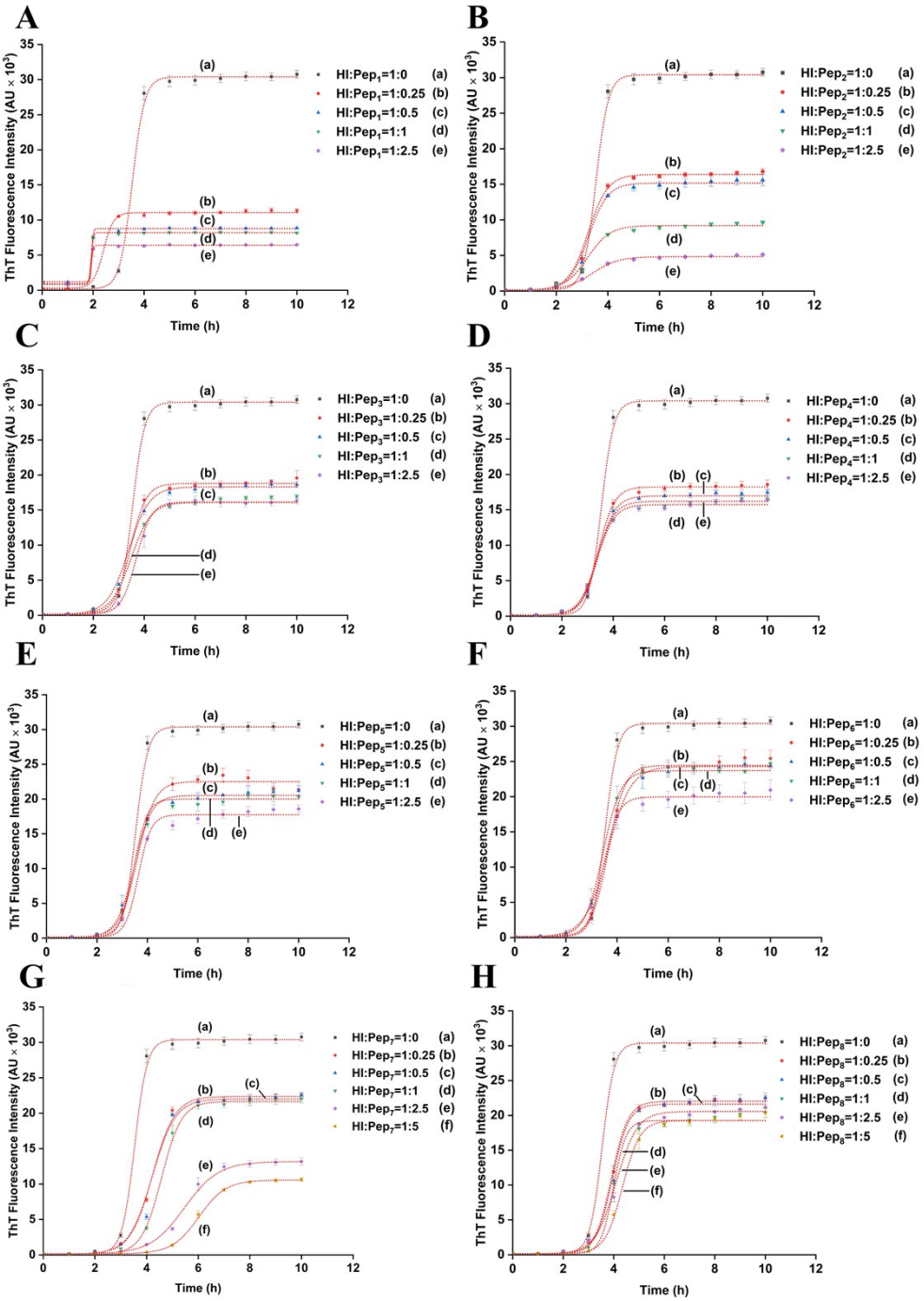


Fig. S1. MS spectra of all fourteen synthesized peptides showing respective masses.


A
ThT Fluorescence Intensity (AU × 10³)
Time (h)
HI:Pep1=1:0 (a)
HI:Pep1=1:0.25 (b)
HI:Pep1=1:0.5 (c)
HI:Pep1=1:1 (d)
HI:Pep1=1:2.5 (e)
B
HI:Pep2=1:0 (a)
HI:Pep2=1:0.25 (b)
HI:Pep2=1:0.5 (c)
HI:Pep2=1:1 (d)
HI:Pep2=1:2.5 (e)
C
HI:Pep3=1:0 (a)
HI:Pep3=1:0.25 (b)
HI:Pep3=1:0.5 (c)
HI:Pep3=1:1 (d)
HI:Pep3=1:2.5 (e)
D
HI:Pep4=1:0 (a)
HI:Pep4=1:0.25 (b)
HI:Pep4=1:0.5 (c)
HI:Pep4=1:1 (d)
HI:Pep4=1:2.5 (e)
E
HI:Pep5=1:0 (a)
HI:Pep5=1:0.25 (b)
HI:Pep5=1:0.5 (c)
HI:Pep5=1:1 (d)
HI:Pep5=1:2.5 (e)
F
HI:Pep6=1:0 (a)
HI:Pep6=1:0.25 (b)
HI:Pep6=1:0.5 (c)
HI:Pep6=1:1 (d)
HI:Pep6=1:2.5 (e)
G
HI:Pep7=1:0 (a)
HI:Pep7=1:0.25 (b)
HI:Pep7=1:0.5 (c)
HI:Pep7=1:1 (d)
HI:Pep7=1:2.5 (e)
HI:Pep7=1:5 (f)
H
HI:Pep8=1:0 (a)
HI:Pep8=1:0.25 (b)
HI:Pep8=1:0.5 (c)
HI:Pep8=1:1 (d)
HI:Pep8=1:2.5 (e)
HI:Pep8=1:5 (f)

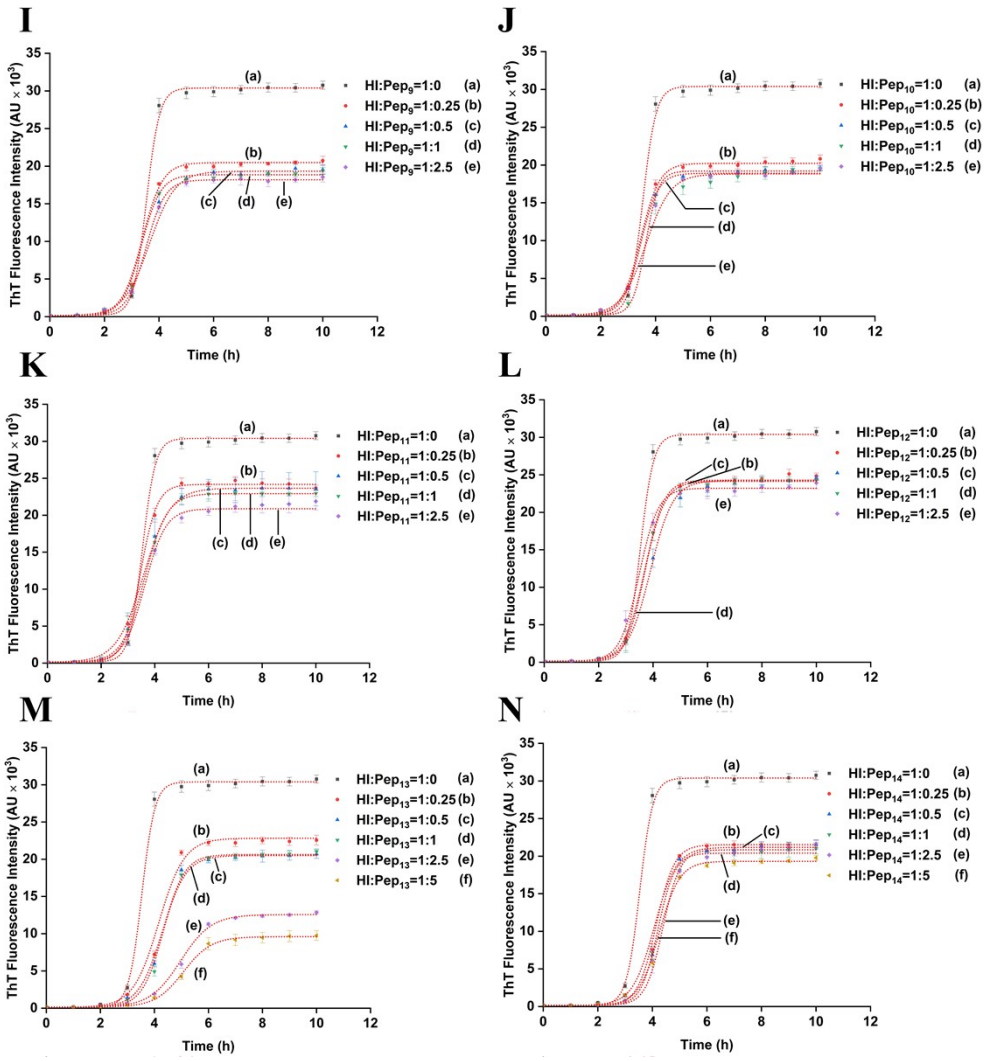


Fig. S2. Time-course ThT fluorescence kinetics of HI at different molar ratios of HI to all fourteen peptides under aggregating conditions of pH 1.6 and 60 ℃. Red dotted lines represent the fitted (modified Boltzmann equation) data. Error bars indicate standard deviations derived from three independent experiments.

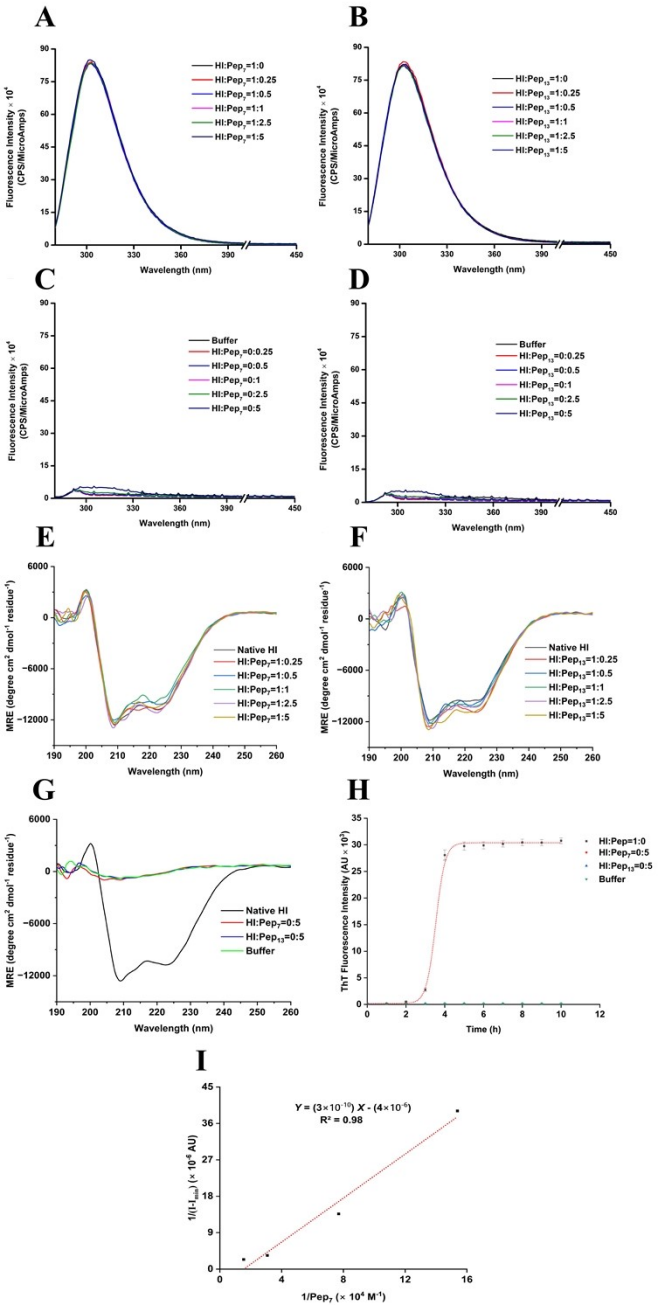


Fig. S3. (A and B). Intrinsic Tyr fluorescence spectra over λ_{em} = 280-450 nm with λ_{ex} = 268 nm of HI with or without different molar ratios of Pep_7 and Pep_{13} mixed and incubated at non-aggregation (pH 1.6, and RT) conditions. **(C and D).** Intrinsic Tyr fluorescence spectra of different molar ratios of only Pep_7 and Pep_{13} without HI. **(E and F).** Circular dichroism spectra for HI with or without incubation having different molar ratios of Pep_7 and Pep_{13} under non-aggregation conditions. **(G and H).** CD **(G)** and ThT kinetics spectra **(H)** of native HI, Pep_7, and Pep_{13} at their highest usage concentrations, and buffer component. **(I).** Fitting of the fluorimetry intensity data of Pep_7 and HI using the Benesi-Hildebrand equation ($y = (3\times10^{-10})x - (4\times10^{-6})$, $R^2 = 0.98$), where the binding coefficient (K_a) was determined to be $(6.28 \pm 0.15) \times10^3\ M^{-1}$.

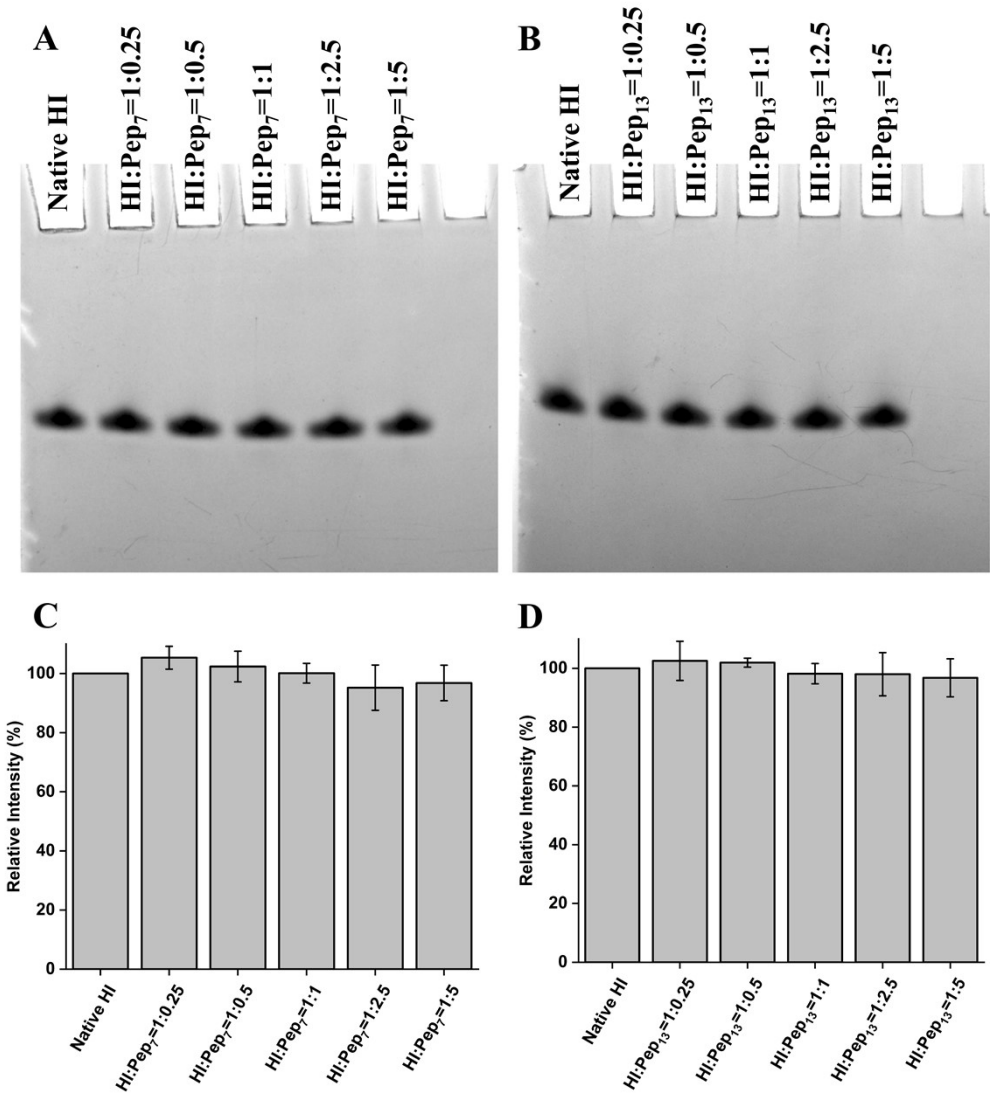


Fig S4. (A and B). Native PAGE bands of HI with or without different molar ratios of Pep_7 and Pep_{13} under non-aggregation conditions. One representative image from the n=3 is shown here. **(C and D).** The relative band intensities of the native PAGE gels were quantified using ImageJ.

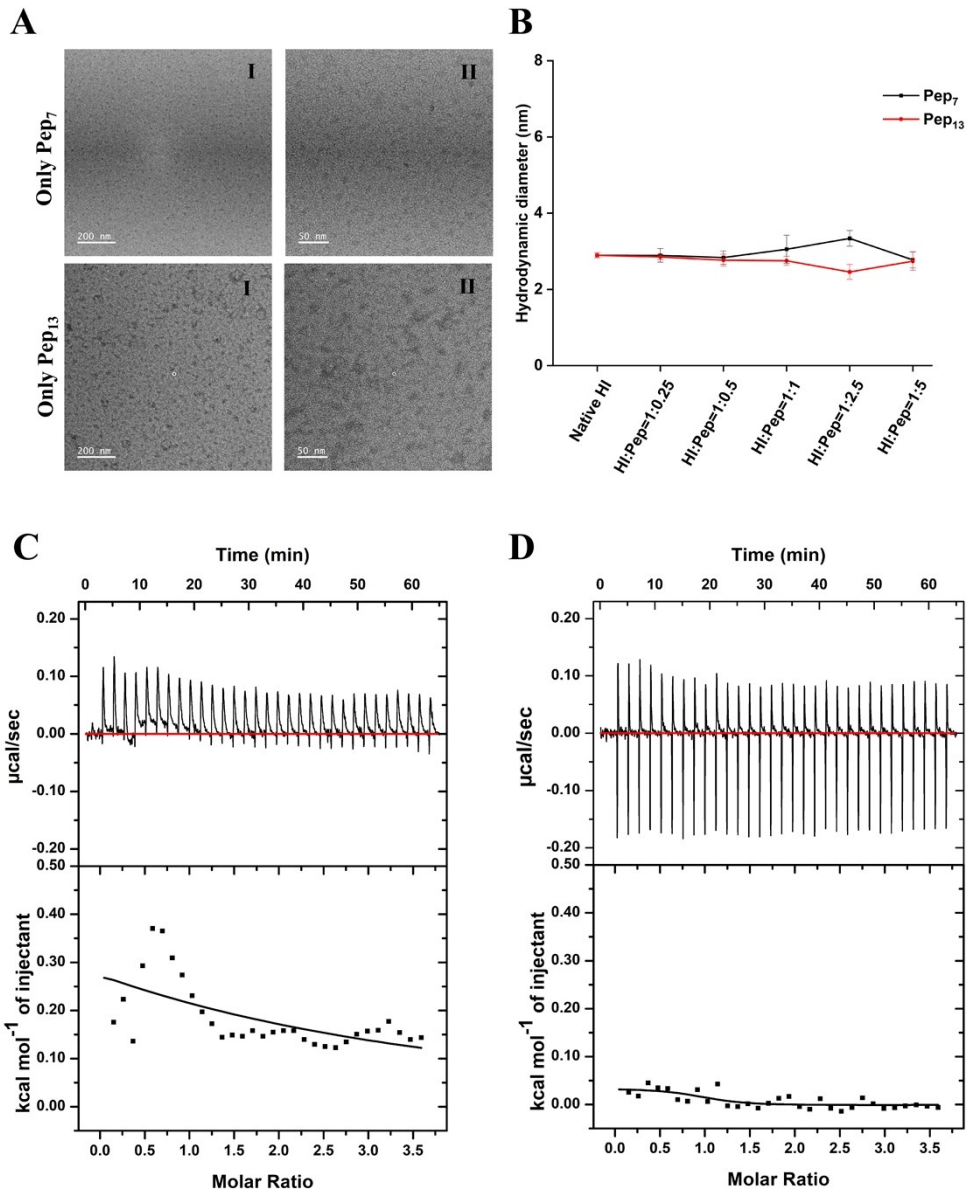


Fig S5. (A). TEM images of only Pep_7 and Pep_{13}. Panels I and II contain images at scales of 200 nm and 50 nm, respectively. **(B).** Hydrodynamic diameter of HI with or without different molar ratios of Pep_7 and Pep_{13} under non-aggregation conditions measured using DLS. **(C and D).** ITC spectra of Pep_1 **(C)** and Pep_2 **(D)** with HI in 32 injections, representing the endothermic nature of the interaction for Pep_1.

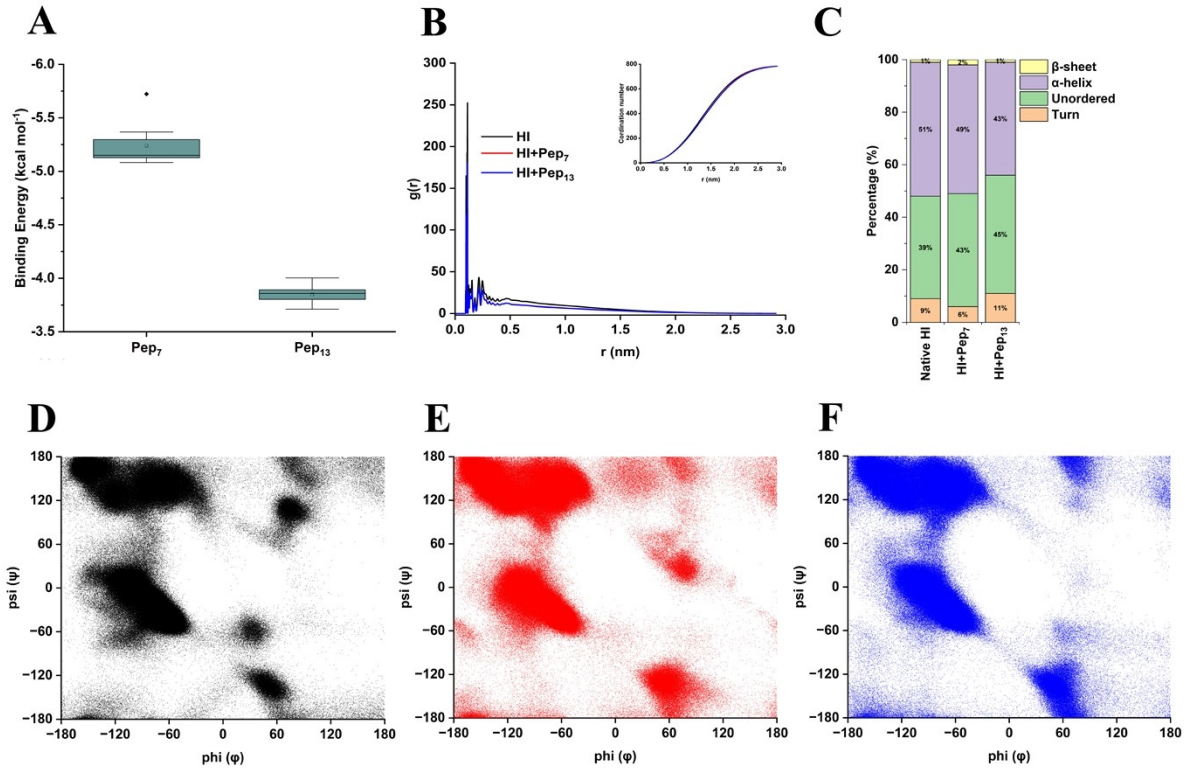


Fig. S6. (A). Binding energy scores of Pep_7 and Pep_{13} with monomeric HI obtained through Autodock Vina. **(B).** Radial distribution function (RDF) and corresponding coordination number (inset) obtained from the MD simulation for HI, HI+Pep_7, and HI+Pep_{13}, indicating no significant change in the presence of the peptides. **(C).** Secondary structural analysis of HI, HI+Pep_7, and HI+Pep_{13}, obtained from the MD trajectories. **(D-F).** Ramachandran plot obtained from the MD simulation of HI, HI+Pep_7, and HI+Pep_{13}.

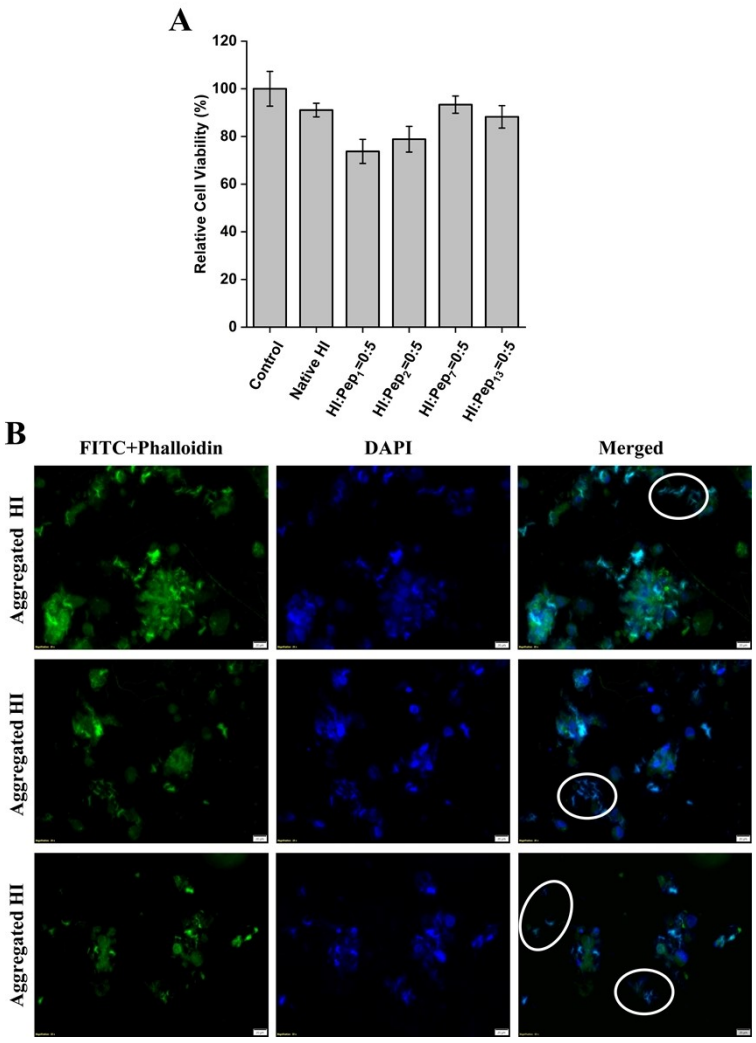


Fig. S7. (A). MTT assay representing the cell viability of HepG2 cells incubated with only Pep_1, Pep_2, Pep_7 and Pep_{13}. **(B).** HepG2 cells incubated with aggregated HI samples, stained with FITC-Phalloidin and nuclear stain DAPI, represented at a scale bar of 20 µm. Aggregated HI samples were marked with white circles.

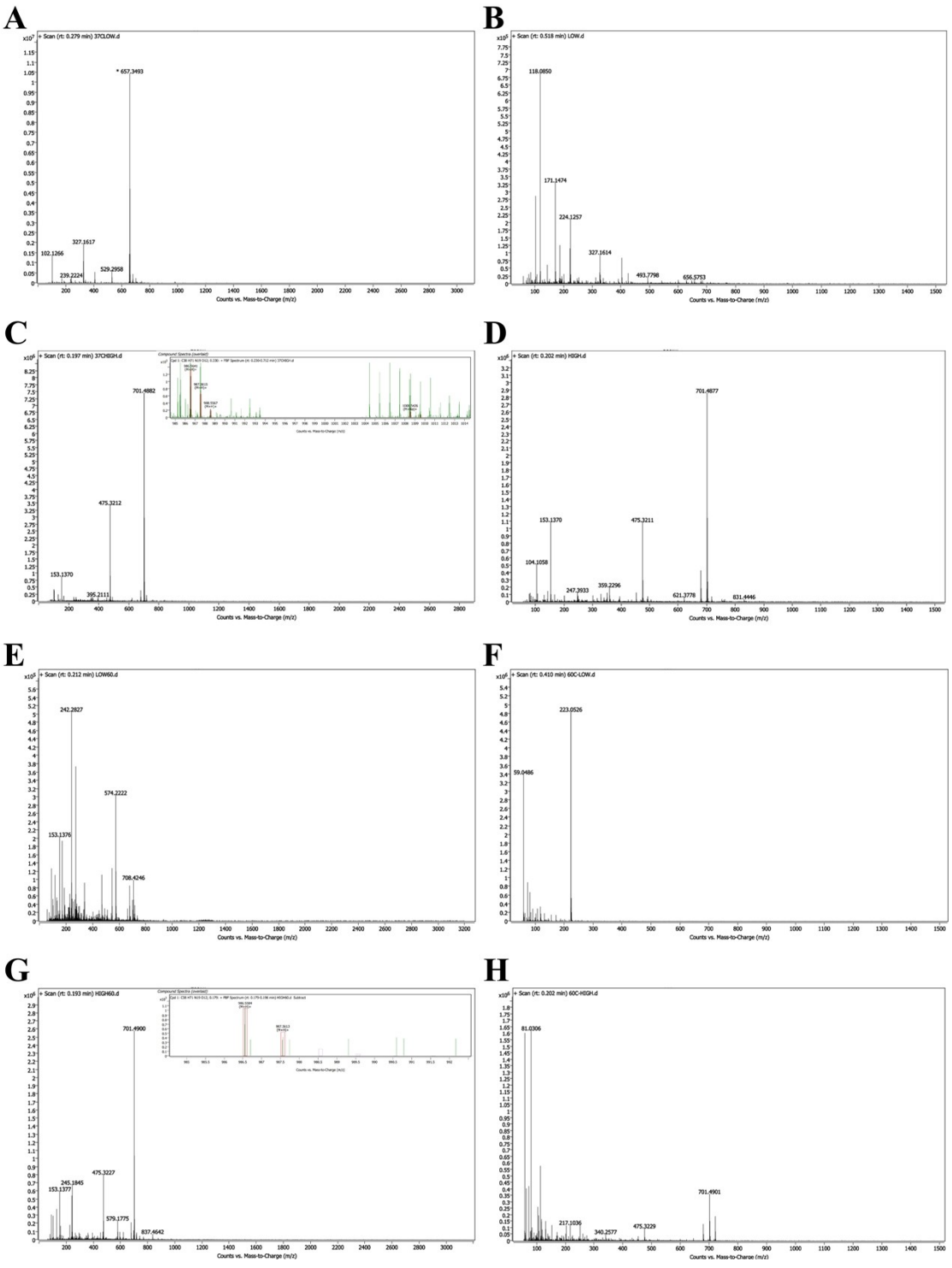


Fig. S8. The degradation pattern of Pep_7, observed using HR-MS under various conditions over 3 and 7 days. Temperature 37 ℃ and pH 1.6: for day 3 **(A)**, and day 7 **(B)**. Temperature 37 ℃ and pH 7.4: for day 3 **(C)** and day 7 **(D)**. Temperature 60 ℃ and pH 1.6: for day 3 **(E)**, and day 7 **(F)**. Temperature 60 ℃ and pH 7.4: for day 3 **(G)** and day 7 **(H)**. The inset figures in **(C) and (G)** represent the target screening of Pep_7 ($C_{38}H_{71}N_{19}O_{12}$) in the HR-MS spectra, representing a peak at 986.5 Da, corresponding to the $M+H^+$ peak.

Table S1. The sequence of all fourteen peptides and other theoretical characteristics predicted using www.pep-calc.com, www.peptide2.com, and the Bachem peptide calculator. LC-MS reported the mass of the fourteen peptides.

Sl. No.	Peptide sequence	Theoretical molecular mass (Da)	Theoretical hydrophobicity	Theoretical basicity and acidity	Observed molecular mass (Da)
1	ELLVDLL (original sequence) **(Pep_1)**	813.48	71.43%	Acidic: 28.57% Basic: 0%	$M+H^+$ = 814.75
2	ELLVDLLA$^{\beta}$ (Ester replaced with βA) **(Pep_2)**	884.52	75%	Acidic: 25% Basic: 0%	$M+H^+$ = 885.83
	D-Leucine replaced with Arginine (R)				
3	ERRVDLL **(Pep_3)**	899.52	42.86%	Acidic: 28.57% Basic: 28.57%	M = 900.67 $(M+2H^+)/2$ = 451.08
4	ERRVDLLA$^{\beta}$ **(Pep_4)**	970.56	50%	Acidic: 25% Basic: 25%	M = 971.67 $(M+2H^+)/2$ = 486.67
5	ELLVDRR **(Pep_5)**	899.52	42.86%	Acidic: 28.57% Basic: 28.57%	M = 900.67 $(M+2H^+)/2$ = 451.00
6	ELLVDRRA$^{\beta}$ **(Pep_6)**	970.56	50%	Acidic: 25% Basic: 25%	M = 971.58 $(M+2H^+)/2$ = 486.50
7	ERRVDRR **(Pep_7)**	985.55	14.29%	Acidic: 28.57% Basic: 57.14%	M = 986.83 $(M+2H^+)/2$ = 494.08
8	ERRVDRRA$^{\beta}$ **(Pep_8)**	1056.59	25%	Acidic: 25% Basic: 50%	M = 1057.83 $(M+2H^+)/2$ = 529.58
	D-Leucine replaced with Lysine (K)				
9	EKKVDLL **(Pep_9)**	843.51	42.86%	Acidic: 28.57% Basic: 28.57%	M = 844.75 $(M+2H^+)/2$ = 423.17
10	EKKVDLLA$^{\beta}$ **(Pep_{10})**	914.54	50%	Acidic: 25% Basic: 25%	M = 915.83 $(M+2H^+)/2$ = 458.67
11	ELLVDKK **(Pep_{11})**	843.51	42.86%	Acidic: 28.57% Basic: 28.57%	M = 844.58 $(M+2H^+)/2$ = 423.00
12	ELLVDKKA$^{\beta}$	914.54	50%	Acidic: 25% Basic: 25%	M = 915.83

	(Pep_{12})				$(M+2H^+)/2 = 458.75$
13	EKKVDKK **(Pep_{13})**	873.53	14.29%	Acidic: 28.57% Basic: 57.14%	M = 874.75 $(M+2H^+)/2 = 438.17$
14	$EKKVDKKA^{\beta}$ **(Pep_{14})**	944.57	25%	Acidic: 25% Basic: 50%	M = 945.75 $(M+2H^+)/2 = 473.58$

Table S2. Lag time, rate, and the ThT intensity maxima of all fourteen peptides at four different concentrations.

Name	HI:Pep=1:0.25			HI:Pep=1:0.5			HI:Pep=1:1			HI:Pep=1:2.5		
	t_{lag} (h)	Rate (h^{-1})	I_{max} ($AU \times 10^3$)	t_{lag} (h)	Rate (h^{-1})	I_{max} ($AU \times 10^3$)	t_{lag} (h)	Rate (h^{-1})	I_{max} ($AU \times 10^3$)	t_{lag} (h)	Rate (h^{-1})	I_{max} ($AU \times 10^3$)
Pep_1	1.48 ± 0.12	3.91 ± 0.4	11.10 ± 0.20	1.10 ± 0.02	4.24 ± 0.4	8.78 ± 0.06	1.19 ± 0.08	5.45 ± 0.9	8.14 ± 0.07	1.15 ± 0.05	5.42 ± 0.3	6.43 ± 0.03
Pep_2	2.63 ± 0.10	2.96 ± 0.1	16.49 ± 0.24	2.62 ± 0.15	2.84 ± 0.1	15.17 ± 0.76	2.30 ± 0.07	2 ± 0.9	9.38 ± 0.19	2.02 ± 0.18	1.47 ± 0.2	5 ± 0.36
Pep_3	2.83 ± 0.02	3.25 ± 0.5	18.95 ± 0.43	2.65 ± 0.07	2.37 ± 0.1	18.51 ± 0.39	2.67 ± 0.02	2.37 ± 0.1	16.55 ± 0.28	3.17 ± 0.1	3.34 ± 0.6	16.44 ± 0.27
Pep_4	2.82 ± 0.05	3.12 ± 0.1	18.3 ± 0.51	2.83 ± 0.06	3.08 ± 0.2	17.28 ± 0.20	2.54 ± 0.07	2.36 ± 0.2	16.24 ± 0.28	2.59 ± 0.04	2.49 ± 0.1	16.02 ± 0.22
Pep_5	2.98 ± 0.10	3.08 ± 0.3	22.2 ± 0.55	2.67 ± 0.14	2.55 ± 0.2	20.77 ± 0.46	2.78 ± 0.01	2.63 ± 0.2	20.30 ± 0.44	2.78 ± 0.20	2.68 ± 0.7	18.10 ± 0.64
Pep_6	2.91 ± 0.05	2.64 ± 0.2	25.12 ± 0.71	2.75 ± 0.11	2.29 ± 0.1	24.33 ± 0.31	2.79 ± 0.15	2.94 ± 0.2	24.12 ± 0.30	2.72 ± 0.22	3.23 ± 0.8	20.28 ± 1.43
Pep_7	3.51 ± 0.05	3.01 ± 0.2	22.23 ± 0.37	3.69 ± 0.04	2.95 ± 0.1	22.28 ± 0.23	3.89 ± 0.05	2.87 ± 0.1	21.83 ± 0.58	4.42 ± 0.01	1.89 ± 0.1	13.22 ± 0.46
Pep_8	3.35 ± 0.09	3.35 ± 0.3	22.11 ± 0.28	3.37 ± 0.08	3.1 ± 0.1	22.17 ± 0.56	3.30 ± 0.18	2.91 ± 0.3	19.86 ± 0.55	3.31 ± 0.06	2.43 ± 0.1	20.72 ± 0.09
Pep_9	2.79 ± 0.04	3.04 ± 0.1	20.48 ± 0.22	2.91 ± 0.07	2.94 ± 0.5	19.3 ± 0.35	2.75 ± 0.13	2.94 ± 0.3	18.96 ± 0.22	2.9 ± 0.05	2.9 ± 0.2	18.26 ± 0.50
Pep_{10}	2.85 ± 0.04	3.12 ± 0.1	20.33 ± 0.49	2.83 ± 0.03	2.91 ± 0.2	19.28 ± 0.27	2.65 ± 0.06	3.02 ± 0.5	18.99 ± 0.43	2.82 ± 0.01	2.59 ± 0.1	18.97 ± 0.10
Pep_{11}	2.78 ± 0.12	2.95 ± 0.1	24.31 ± 0.16	2.78 ± 0.28	2.57 ± 0.6	23.59 ± 1.99	2.75 ± 0.25	2.41 ± 0.4	22.90 ± 0.30	2.73 ± 0.19	2.26 ± 0.4	21.43 ± 1.07
Pep_{12}	2.97 ± 0.08	2.75 ± 0.1	24.57 ± 0.41	2.95 ± 0.16	2.17 ± 0.3	24.32 ± 0.29	3.03 ± 0.26	3.02 ± 0.6	24.20 ± 0.34	2.69 ± 0.12	2.56 ± 0.1	23.43 ± 0.14
Pep_{13}	3.56 ± 0.02	3.10 ± 0.1	22.48 ± 0.45	3.59 ± 0.01	2.92 ± 0.1	20.56 ± 0.57	3.69 ± 0.06	2.78 ± 0.1	20.84 ± 0.43	4 ± 0.03	1.92 ± 0.1	12.73 ± 0.18
Pep_{14}	3.54 ± 0.06	3.13 ± 0.2	21.61 ± 0.41	3.55 ± 0.05	3.06 ± 0.2	21.27 ± 0.52	3.48 ± 0.06	2.57 ± 0.3	20.81 ± 0.85	3.54 ± 0.2	2.55 ± 0.4	21.19 ± 0.54

Table S3. ThT I_{max}, lag time, and rate of aggregation of HI containing different molar ratios of Pep_7 and Pep_{13}.

Sample	Relative inhibition in ThT_{max} with no peptide control (%)	HI fibrillation lag time (h)	Relative increase in lag time with no peptide control (%)	Rate of fibrillation (h^{-1})	Relative decrease in rate with no peptide control (%)
HI:Pep=1:0	NA	2.81 ± 0.02	NA	5.79 ± 0.1	NA
HI:Pep_7=1:0.25	26.67	3.51 ± 0.05	25.06	3.01 ± 0.22	48.02
HI:Pep_7=1:0.5	26.49	3.69 ± 0.04	31.34	2.95 ± 0.12	48.89
HI:Pep_7=1:1	28	3.89 ± 0.05	38.63	2.87 ± 0.15	50.38
HI:Pep_7=1:2.5	56.39	4.42 ± 0.01	57.59	1.89 ± 0.12	67.33
HI:Pep_7=1:5	64.86	4.92 ± 0.07	75.17	1.90 ± 0.11	67.11
HI:Pep_{13}=1:0.25	25.85	3.56 ± 0.02	26.87	3.1 ± 0.11	46.41
HI:Pep_{13}=1:0.5	32.18	3.59 ± 0.01	27.79	2.92 ± 0.04	49.41
HI:Pep_{13}=1:1	31.26	3.69 ± 0.06	31.64	2.75 ± 0.1	52.43
HI:Pep_{13}=1:2.5	58.01	4 ± 0.03	42.61	1.92 ± 0.06	66.69
HI:Pep_{13}=1:5	68.04	4.08 ± 0.03	45.50	2.02 ± 0.1	65.01

Table S4. Residue-specific binding interaction between Pep_7 and Pep_{13} and HI.

		Interacting Residues	
	Binding energy (kJ mol^{-1})	**H-bonds**	**Electrostatic interactions**
Pep_7	-23.94	Cluster 1: Gly^{A1}, Ile^{A2}, Val^{A3}, Gln^{A5}	Gly^{A1}, Tyr^{A19}
		Cluster 2: Asn^{A18}, Tyr^{A19}, Cys^{A20}, Asn^{A21}	
		Cluster 3: Thr^{B27}, Pro^{B28}, Lys^{B29}	
Pep_{13}	-16.75	Cluster 1: Gly^{A1}, Ile^{A2}, Glu^{A4}	Gly^{A1}, Glu^{A4}, Tyr^{B26}
		Cluster 2: Tyr^{A19}, Cys^{A20}, Asn^{A21}	
		Cluster 3: Phe^{B25}, Tyr^{B26}, Thr^{B27}, Lys^{B29}	

Table S5. Binding energy components of peptides with HI obtained from gmx_mmpbsa of the MD simulation.

Sample	ΔG_{gas} (kJ mol^{-1})		ΔG_{solv} (kJ mol^{-1})		ΔG_{total} (kJ mol^{-1})
	ΔE_{vDW} (kJ mol^{-1})	ΔE_{Elec} (kJ mol^{-1})	ΔE_{polar} (kJ mol^{-1})	$\Delta E_{non-polar}$ (kJ mol^{-1})	
Pep_7 (ERRVDRR)	-74.81	-1355.36	1338.46	-16.48	-108.20
Pep_{13} (EKKVDKK)	-63.64	-1078.22	1097.97	-11.67	-55.61

Table S6. Quantitative morphometric analysis of HepG2 cells treated with native HI, HI+Pep_7, HI+Pep_{13}, and aggregated HI, using ImageJ. Data represent mean ± SD for cell area and nuclear area.

Sample	Cell area (µm^2)	Nuclear area (µm^2)
Native HI	1665.2 ± 301.8	362.1 ± 52.3
Aggregated HI	379.43 ± 28.54	86 ± 12.8
HI+Pep_7	1904 ± 278.3	400.8 ± 32.2
HI+Pep_{13}	1521.7 ± 156.4	391.4 ± 77.5