S1

Supporting Information

Homotrimerization Approach in the Design of Thrombospondin-1 Mimetic Peptides with Improved Potency in Triggering Regulated Cell Death of Cancer Cells

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S2

Electronic Supplementary Information (ESI)

1) **Synthesis and characterization of the peptides**
2) **Stability studies**
3) **Structural analyses**
4) **Binding affinity measurements**
5) **Cell culture**
6) **Cell death induction and inhibition**
7) **Cell viability evaluation through impedance**
8) **Molecular formula strings**

1. Synthesis and characterization of the peptides

- *General methods:*

Chemicals: All commercial chemicals and solvents were reagent grade and were used without further purification unless otherwise specified. All reactions except those in aqueous media were carried out with the use of standard techniques for the exclusion of moisture. All reactions were performed under argon or nitrogen in oven-dried glassware using anhydrous solvents and standard syringe techniques. Peptide synthesis transformations and washes were performed at room temperature. All Fmoc carbamate protected amino acid derivatives, coupling reagents (PyOxim/Oxyma Pure), Fmoc-Rink Amide (200-400 mesh, loading 0.62 mmol/g) and 2-CTC resin (100-200 mesh, loading 1.6 mmol/g) were purchased from *Iris Biotech* (Marktredwitz, Germany). Reagents such as DIEA, piperidine, DMF, IPA, Ac_2O, MeOH, TFA and TIS were obtained from *Sigma-Aldrich* (Saint Louis, USA). Compounds molecular weights were calculated using ChemBioDraw® Ultra 12. All final products were of > 95% purity unless otherwise indicated (determined by analytical reverse phase LC-MS). Analytical data are given in **Table S1**.

Analytics: Two methods were conducted for LC-MS analysis. *Method A*: analytical HPLC was conducted on a X-Select CSH C18 XP column (30 × 4.6 mm id, 2.5 µm) eluting with 0.1% formic acid in water (solvent A) and 0.1% formic acid in acetonitrile (solvent B), using the following elution gradient 0 - 3.2 min: 0% to 50% B, 3.2 - 4 min 100% B, at a flow rate of 1.8 mL/min at 40 °C. The mass spectra (MS) were recorded on a *Waters* ZQ mass spectrometer using electrospray positive ionisation [ES+ to give $(MH)^+$ molecular ions] or electrospray negative ionisation [ES- to give $(MH)^-$ molecular ions] modes. The cone voltage was 20 V. *Method B*: analytical HPLC was conducted on a X-Select CSH C18 XP column (30 × 4.6 mm id, 2.5 µm) eluting with 0.1% formic acid in water (solvent A) and 0.1% formic acid in acetonitrile (solvent B), using the following elution gradient 0 - 3.2 min: 5% to 100% B, 3.2 - 4 min: 100% B, at a flow rate of 1.8 mL/min at 40 °C. The mass spectra (MS) were recorded on a *Waters* ZQ mass spectrometer using electrospray positive ionisation [ES+ to give $(MH)^+$ molecular ions] or electrospray negative ionisation [ES^- to give $(MH)^-$ molecular ions] modes. The cone voltage was 20 V.

Purification: Preparative scale purification of peptides was performed by reverse phase HPLC on a *Waters* system consisted of a quaternary gradient module (Water 2535) and a dual wavelength UV/Visible Absorbance detector (Waters 2489), piloted by *Empower Pro 3* software using the following columns: preparative *Macherey-Nagel* column (Nucleodur HTec, C18, 250 × 16 mm id, 5 µm, 110 Å) and preparative *Higgins Analytical* column (Proto 200, C18, 150 × 20 mm id, 5 µm, 200 Å) at a flow rate of 14 mL/min and 20 mL/min respectively. Small-scale crudes (< 30 mg) were purified using semi-preparative *Ace* column (Ace 5, C18, 250 × 10 mm id, 5 µm, 300 Å) at a flow rate of 5 mL/min. Purification gradients were chosen to get a ramp of approximately 1% solution B per minute in the interest area and UV detection was done at 220 nm and 280 nm. Peptide fractions from purification were analyzed by LC-MS (method A or B depending of retention time) or by analytical HPLC on a *Dionex* system consisted of an automated LC system (Ultimate 3000) equipped with an auto sampler, a pump block composed of two ternary gradient pumps and a dual wave-length detector, piloted by *Chromeleon* software. All LC-MS or HPLC analyses were performed on C18 columns. The pure fractions were gathered according to their purity and then freeze-dried using an Alpha 2/4 freeze dryer from *Bioblock Scientific* to get the expected peptide as a white powder. Final

peptide purity (> 95%) of the corresponding pooled fractions was checked by LC-MS using method A.

- *Manual loading of the first amino acid:*

Solid-phase peptide syntheses were performed in polypropylene Torviq syringes (10 or 20 mL) fitted with a polyethylene porous disc at the bottom and closed with an appropriate piston. Solvent and soluble reagents were removed through back and forth movements. The 2-CTC resin was previously swelled in strictly anhydrous DCM (distilled) for 2 h. Side-chain protected Fmoc-Aa-OH (0.30 mmol, 1eq.) was coupled to 2-CTC resin (400 mg, loading 1.6 mmol/g) in the presence of DIEA (1.2 mmol, 4 eq.) in DCM (4 mL). The unreacted sites on the resin were capped by washing with a mixture of DCM/MeOH/DIEA (17:2:1) repeated 3 times. Thus loading was reduced to 0.80 mmol/g for optimal peptide growth. In the case of Rink Amide resin, swollen in DCM was done similarly in 2 h (500 mg, loading 0.62 mmol/g). However, first coupling was directly performed with protected Fmoc-Aa-OH (1.2 mmol, 4 eq.), PyOxim (1.2 mmol, 4 eq.), Oxyma Pure (1.2 mmol, 4 eq.) and DIEA (2.4 mmol, 8 eq.) without loading decreasing.

- *Manual solid phase peptide synthesis:*

In all syntheses the scale was 0.30 mmol. Fmoc group was split off by treatment with piperidine/DMF (1:4) (1 × 1 min, 1 × 10 min). Washing steps between deprotection and coupling were carried out with DMF (3 × 1 min), IPA (3 × 1 min) and DMF (3 × 1 min). Activation step was carried out with Fmoc-Aa-OH (1.2 mmol, 4 eq.), PyOxim (1.2 mmol, 4 eq.) as coupling agent, Oxyma Pure (1.2 mmol, 4 eq.) as auxiliary nucleophile, and DIEA (2.4 mmol, 8 eq.) as base. The activated amino acid is then transferred to the resin where the coupling was performed for 1 to 18 h. Supported coupling reactions were monitored by classical Kaiser test (solution kit from *Sigma-Aldrich*). When elongation of the peptide chain was completed, a MeOH washing step was added after final *N*-terminal Fmoc removal for complete shrinkage of the resin under vacuum.

- *Site-selective N-Methylation of peptide backbone:*

Residue was *N*-methylated on solid-phase through Kessler's methodology: first, the free amino functionality was protected and activated with the *o*-nitrobenzenesulfonyl (*o*-NBS) group, then *N*-methylated using 1,8-diazabicyclo[5,4,0]undec-7-ene (DBU) and dimethylsulfate (DMS), and finally deprotected (removal of *o*-NBS) by treating the resin with *ß*-mercaptoethanol and DBU.

***o*-NBS Activation:** A solution of *o*-NBS-Cl (4 eq.) and collidine (10 eq.) in NMP was added to the resin-bound free amine peptides and shaken for 15 min at room temperature. The resin was washed with NMP (5×).

***N*-Methylation with DBU and DMS:** A solution of DBU (3 eq.) in NMP was added to the resin bound *o*-NBS-protected peptides and shaken for 3 min. A solution of dimethylsulfate (10 eq.) in NMP was then added to the reaction mixture and shaken for 2 min. The resin was filtered off, washed once with NMP and the *N*-methylation procedure repeated once more. The resin was washed with NMP (5×).

***o*-NBS Deprotection:** The resin bound N^{α}-methyl-N^{α}-*o*-NBS-peptides was treated with a solution of *ß*-mercaptoethanol (10 eq.) and DBU (5 eq.) in NMP for 5 min. The deprotection procedure was repeated once more and the resin was washed with NMP (5×).

- *Homotrimerization by solution phase click chemistry (CuAAC):*

Typically, pure PKT16-N_3 TFA salts (47,1 mg, 24 µmol, 3.6 eq.) was taken up in 750 µL MeOH that was frozen and put on a vacuum pump to remove gas molecules and then purged with N_2 for at least 30 min. To this mixture was added tripropargylamine (0.94 µL, 6.7 µmol, 1 eq.) in 250 µL MeOH, followed by the addition of Tris[(1-benzyl-1H-1,2,3-triazol-4-yl)methyl]amine (TBTA, 21 mg, 40 µmol, 6 eq.) and Tetrakis (acetonitrile)copper(I) hexafluorophosphate ($[(CH_3CN)_4Cu]PF_6$, 78 mg, 203 µmol, 30 eq.). To this mixture was added MeCN dropwise (around 20 drops) to bring everything into solution, and the reaction was allowed to proceed in a round-bottom flask at room temperature under nitrogen flow with constant stirring for

72 h. The sample was quenched with 5 mL H_2O, frozen, and then lyophilized. The dried crude product was re-suspended in 0.1 M EDTA (3×5 mL to rinse the tube), loaded on a 1.6 g zeo prep 90 C18 (part. size 40-63 µm) cartridge, and sequentially eluted with 25 mL H_2O, 1:1 H_2O/MeCN, and MeCN. Each fraction was collected in a round-bottom flask and analyzed by LCMS (method A). The EDTA, H_2O and H_2O/MeCN fractions were concentrated to dryness, re-suspended in H_2O, filtered with 0.22 µm filter and purified as described earlier to obtain $[PKT16]_3$ as a white powder (depending on experiments: 5 – 15% yield).

- *Final side-chain deprotection and cleavage from the resin:*

The crude peptides were treated with the following cleavage cocktail: TFA/H_2O/TIS (95/2.5/2.5, 10 mL). The syringes were shaken for 3 h and then precipitated 3 times using cooled Et_2O (3 × 30 mL), recovered after centrifugations (3 × 5 min, 7800 rpm), diethyl ether was removed (3 times), and then the peptide pellets were dried (under nitrogen flow). The resulting crude peptide was dissolved in aqueous 0.1% (v/v) TFA. Purification was conducted on reversed-phase HPLC Prep C18 column, eluting with 0.1% TFA in water (solvent A) and 0.1% TFA in acetonitrile (solvent B) as described earlier.

Table S1. Analytical data for the all synthesized peptides. Retention times are indicated for LCMS method A. Masses determined by LC-MS (ESI) are also shown.

Peptide	Mw ($g.mol^{-1}$)	*m/z* (ESI)	t_R (min)
5	**1398.8**	**1399.8 $[M+H]^+$**	**1.70**
		700.2 $[M+2H]^{2+}$	
		467.1 $[M+3H]^{3+}$	
		350.6 $[M+4H]^{4+}$	
4	**1398.8**	**1399.8 $[M+H]^+$**	**1.66**
		700.2 $[M+2H]^{2+}$	
		467.1 $[M+3H]^{3+}$	
		350.6 $[M+4H]^{4+}$	
3	**1398.8**	**1399.7 $[M+H]^+$**	**1.74**

		700.1 $[M+2H]^{2+}$	
		467.1 $[M+3H]^{3+}$	
		350.6 $[M+4H]^{4+}$	
2	1398.8	1399.7 $[M+H]^{+}$	1.71
		700.1 $[M+2H]^{2+}$	
		467.1 $[M+3H]^{3+}$	
		350.6 $[M+4H]^{4+}$	
1	1398.8	1399.6 $[M+H]^{+}$	1.61
		700.1 $[M+2H]^{2+}$	
		467.1 $[M+3H]^{3+}$	
		350.6 $[M+4H]^{4+}$	
7	1398.8	1399.7 $[M+H]^{+}$	1.65
		700.1 $[M+2H]^{2+}$	
		467.1 $[M+3H]^{3+}$	
		350.6 $[M+4H]^{4+}$	
6	1398.8	1399.7 $[M+H]^{+}$	1.67
		700.1 $[M+2H]^{2+}$	
		467.1 $[M+3H]^{3+}$	
		350.5 $[M+4H]^{4+}$	
8	1398.8	1399.8 $[M+H]^{+}$	1.67
		700.1 $[M+2H]^{2+}$	
		467.1 $[M+3H]^{3+}$	
		350.6 $[M+4H]^{4+}$	
9	1412.8	1413.8 $[M+H]^{+}$	1.67
		707.1 $[M+2H]^{2+}$	
		471.1 $[M+3H]^{3+}$	
		354.1 $[M+4H]^{4+}$	
10 (PKT16)	1380.8	1381.8 $[M+H]^{+}$	1.69
		691.1 $[M+2H]^{2+}$	
		461.0 $[M+3H]^{3+}$	
		346.0 $[M+4H]^{4+}$	
11	1380.8	691.1 $[M+2H]^{2+}$	1.68
		461.0 $[M+3H]^{3+}$	
		346.0 $[M+4H]^{4+}$	
12	1380.8	691.1 $[M+2H]^{2+}$	1.66
		461.0 $[M+3H]^{3+}$	
		346.0 $[M+4H]^{4+}$	
13	1430.8	1431.8 $[M+H]^{+}$	1.60
		716.1 $[M+2H]^{2+}$	
		477.7 $[M+3H]^{3+}$	
		358.6 $[M+4H]^{4+}$	

14	**1366.7**	**684.1 $[M+2H]^{2+}$**	**1.60**
		456.4 $[M+3H]^{3+}$	
		342.2 $[M+4H]^{4+}$	
15	**1165.5**	**1165.6 $[M+H]^{+}$**	**2.42**
		583.9 $M+2H]^{2+}$	
$[PKHB1]_3$	**4661.1**	**1554.7 $[M+3H]^{3+}$**	**1.99**
		1166.1 $[M+4H]^{4+}$	
		933.2 $[M+5H]^{5+}$	
		777.7 $[M+6H]^{6+}$	
		666.7 $[M+7H]^{7+}$	
		583.5 $[M+8H]^{8+}$	
		518.8 $[M+9H]^{9+}$	
		467.0 $[M+10H]^{10+}$	
$[PKT16]_3$	**4648.8**	**1163.0 $[M+4H]^{4+}$**	**2.10**
		930.7 $[M+5H]^{5+}$	
		775.6 $[M+6H]^{6+}$	
		664.9 $[M+7H]^{7+}$	
		581.9 $[M+8H]^{8+}$	
		517.4 $[M+9H]^{9+}$	
PKHB1-SO	**1400.8**	**1401.6 $[M+H]^{+}$**	**1.50**
		701.1 $[M+2H]^{2+}$	
		467.7 $[M+3H]^{3+}$	
		351.1 $[M+4H]^{4+}$	
PKHB1-SO_2	**1416.8**	**717.1 $[M+2H]^{2+}$**	**1.43**
		473.3 $[M+3H]^{3+}$	
		355.1 $[M+4H]^{4+}$	
SP (Scrambled Peptide)	**1384,8**	**1385.6 $[M+H]^{+}$**	**1.57**
		693.2 $[M+2H]^{2+}$	
		462.4 $[M+3H]^{3+}$	
		347.1 $[M+4H]^{4+}$	

- *<u>HPLC traces of the purified peptide:</u>*

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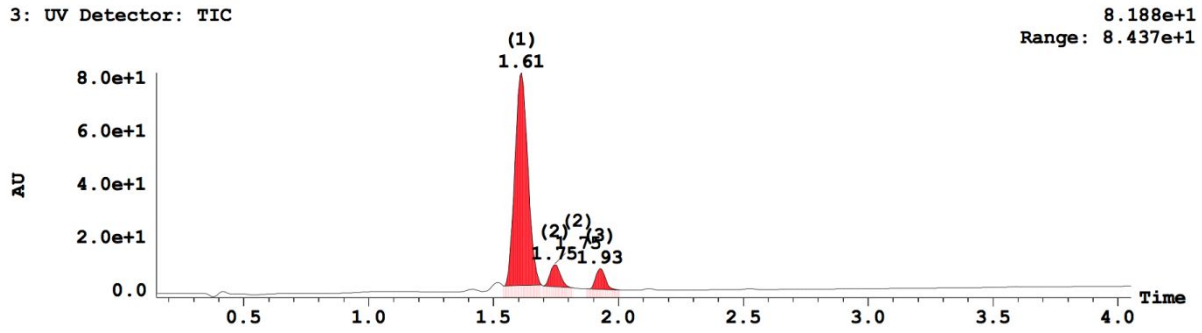

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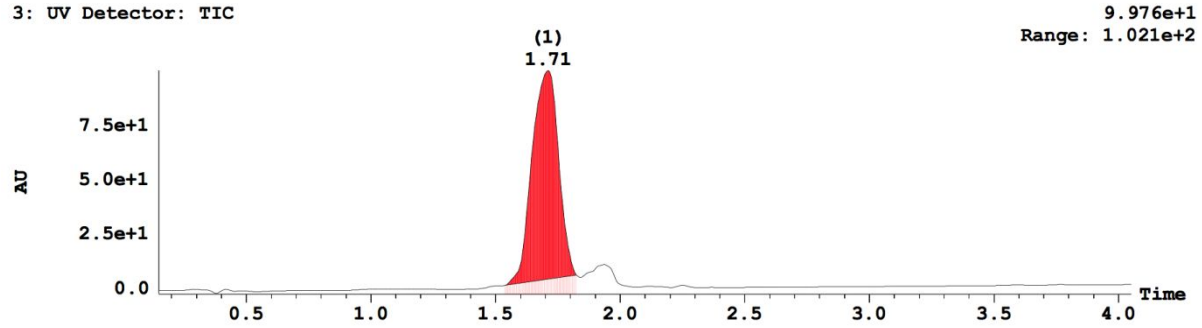

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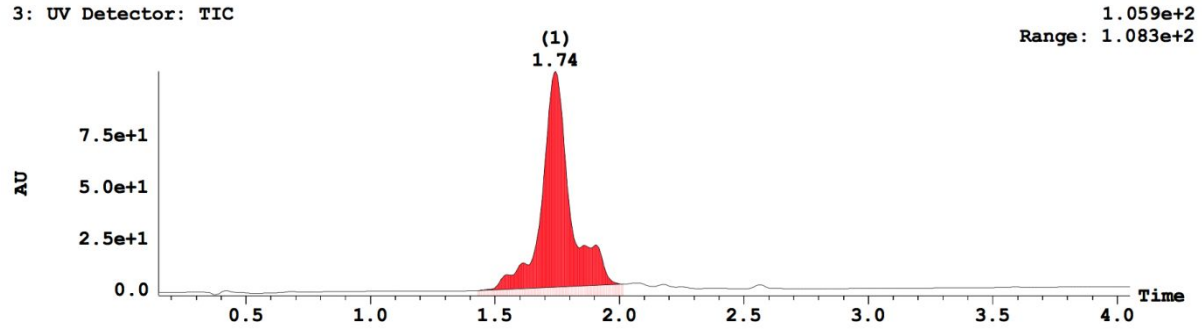

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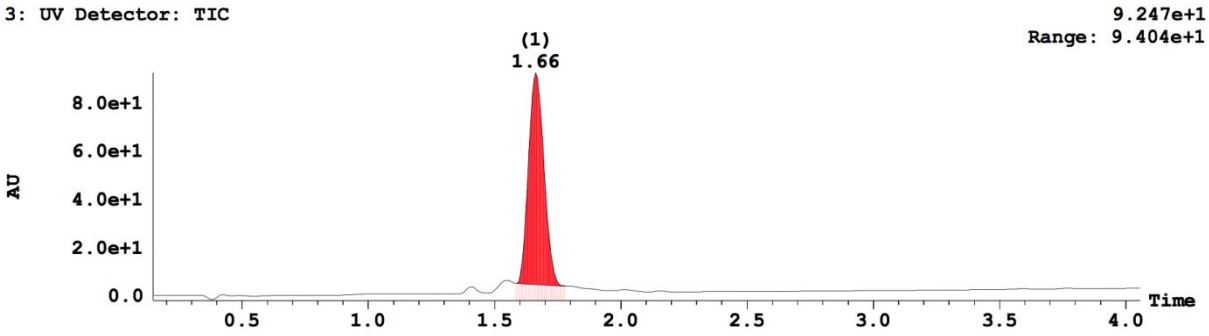

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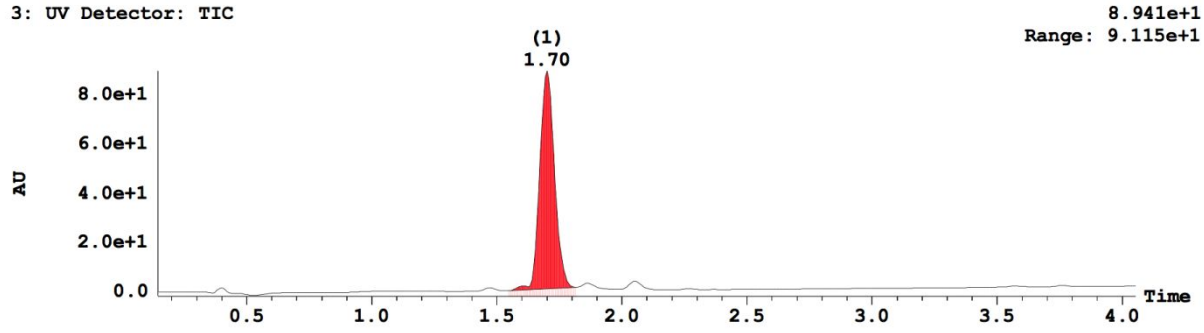

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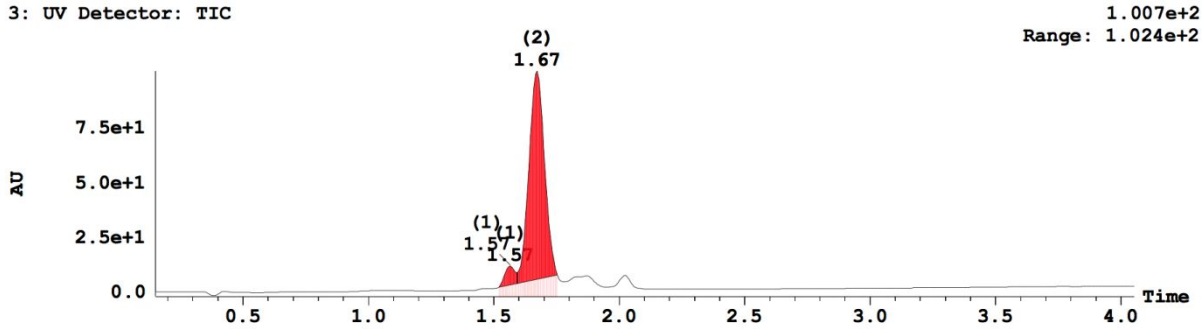

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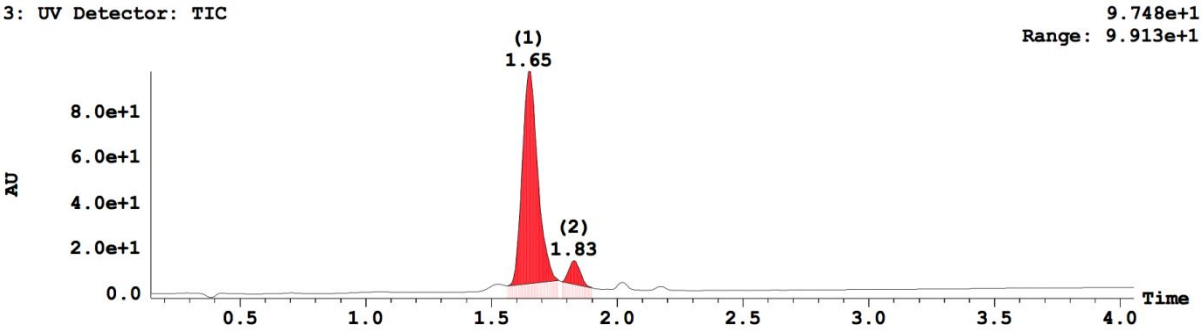

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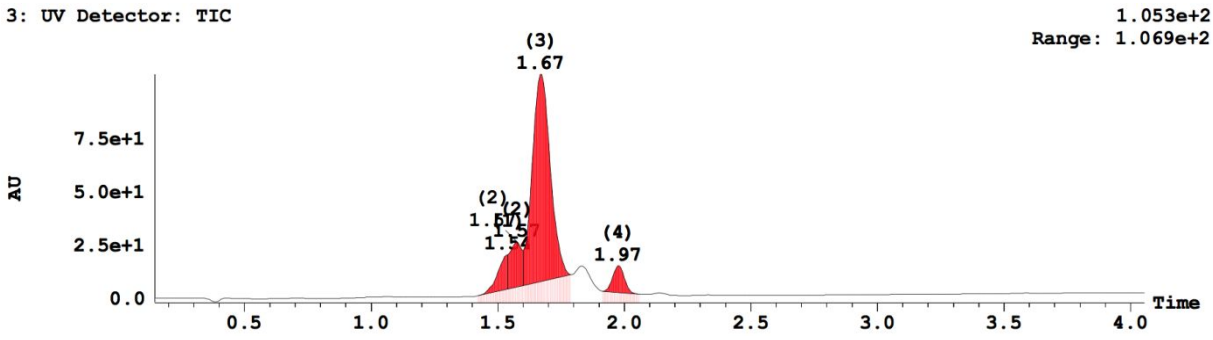

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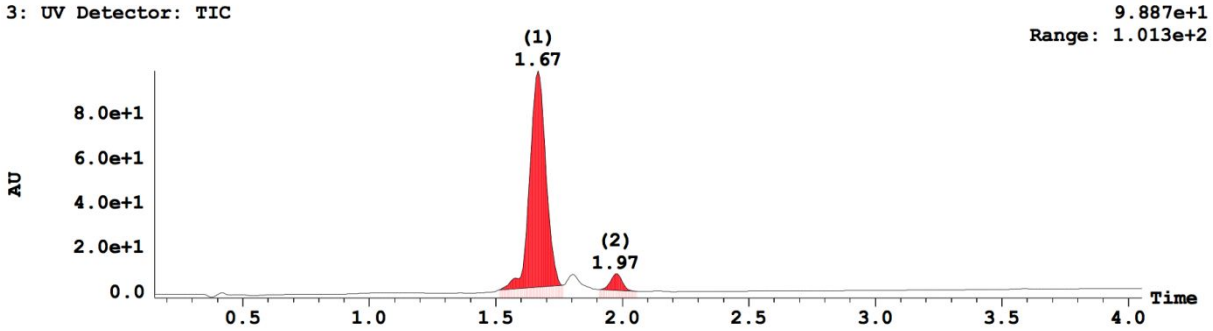

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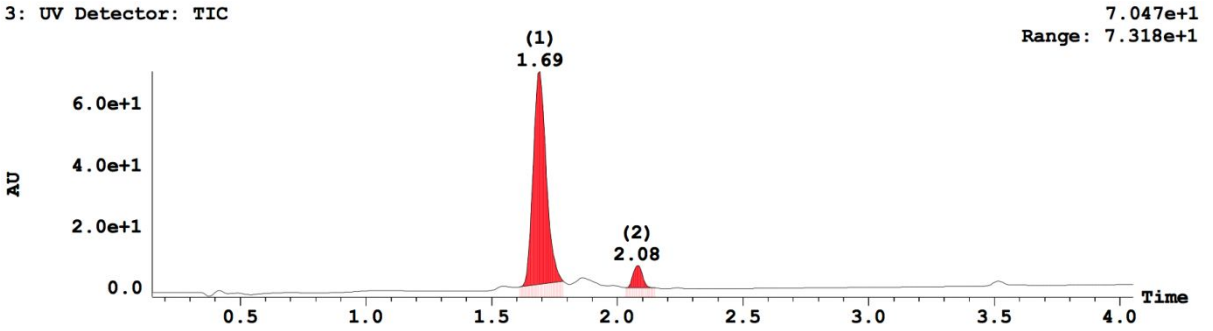

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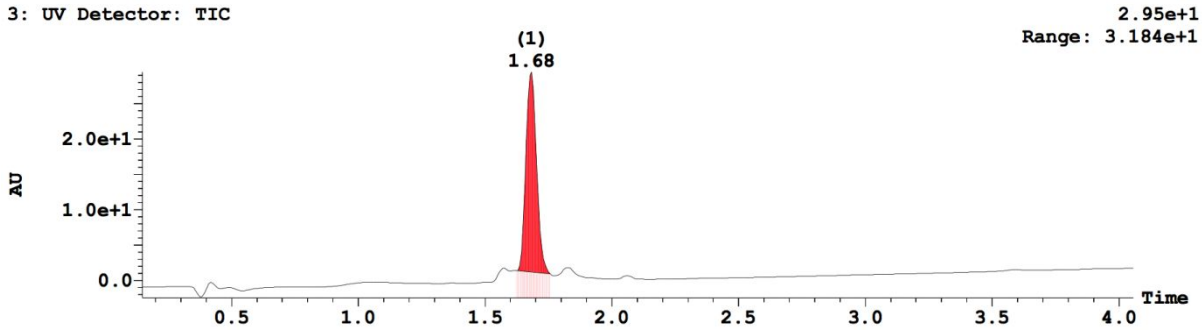

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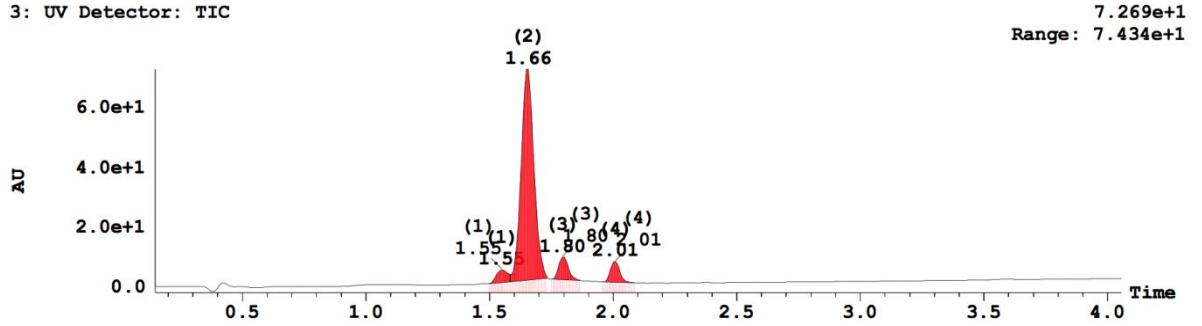

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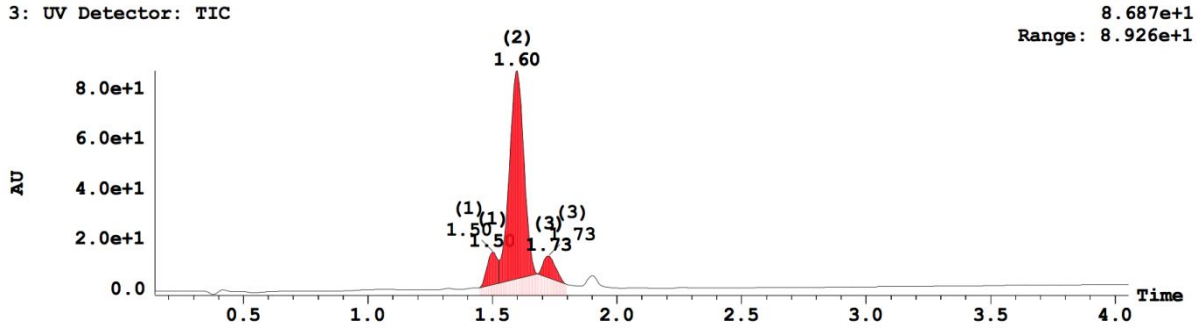

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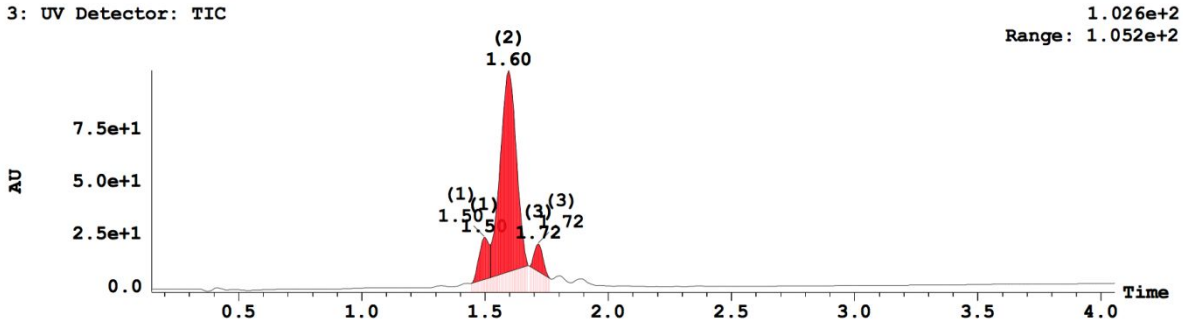

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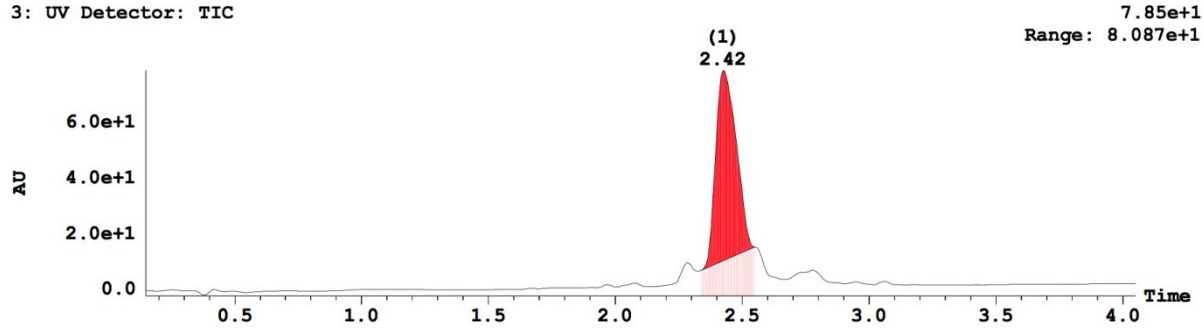

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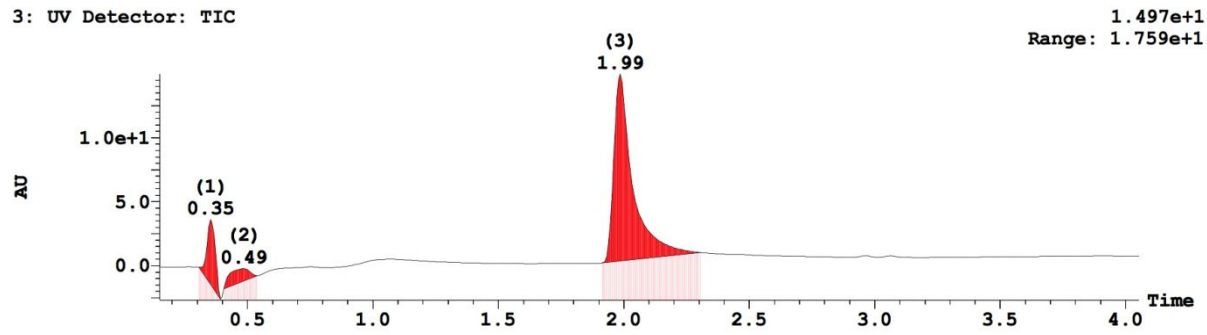
Sample 2 Vial 1,2:20 ID Z4090-2 File Z4090-2 Date 21-Jul-2017 Time 12:13:36 Description TRIMER-HB12



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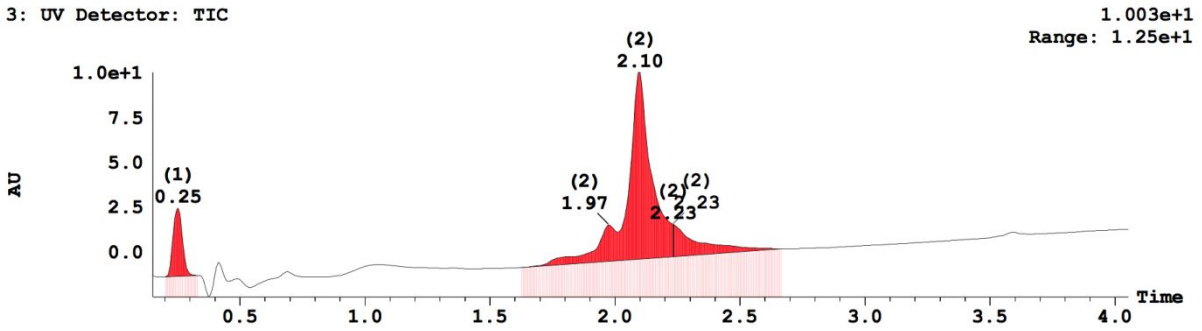

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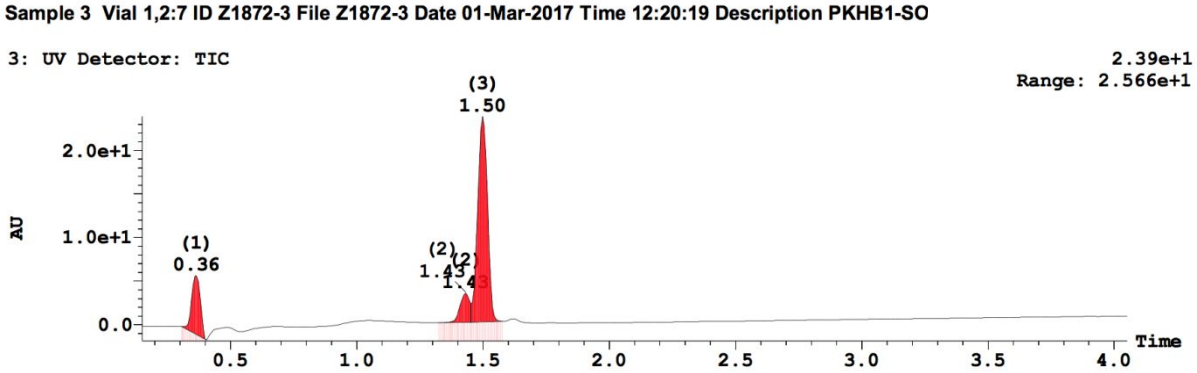

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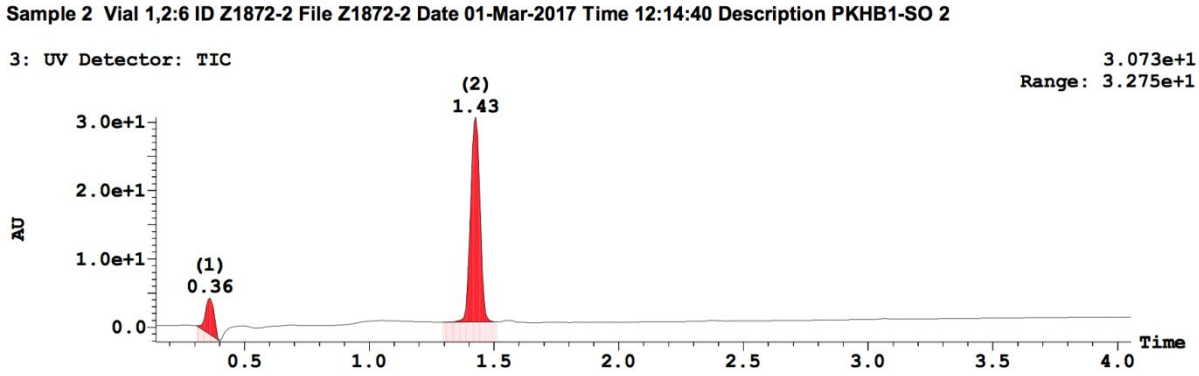
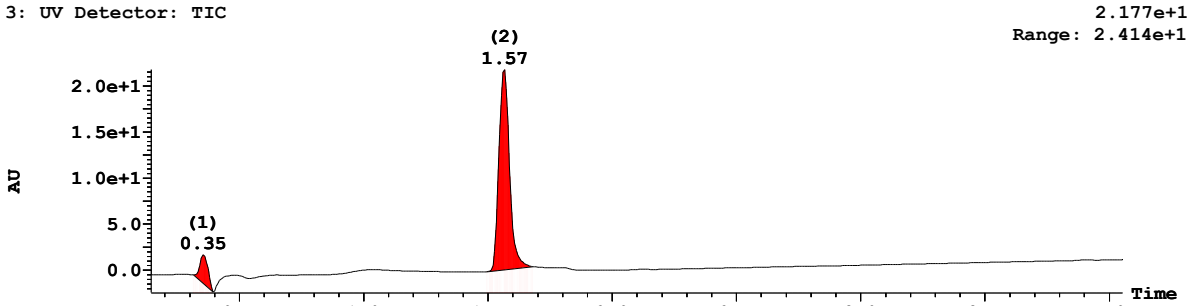


Scramble Peptide



Peak Number	Compound	Time	AreaAbs	Area %Total	Width	Height	Mass Found
1		0.35	1e+005	9.98	0	3e+006	
2		1.57	1e+006	90.02	0	2e+007	

- *Concentration determination of peptide stock solutions:*

The concentration of peptide was determined by absorption spectrometry at 280 nm or by weighing the lyophilized peptide before dissolving in water, or both. For determining the concentration of peptides by absorption spectrometry, theoretical extinction coefficients (ε280) were calculated based on the presence of tryptophan (ε = 5500 $M^{-1}cm^{-1}$) and tyrosine (ε = 1490 $M^{-1}cm^{-1}$). For determining the concentration of peptides by weighing, it was assumed that one trifluoroacetic acid molecule is bound per positive charge of the peptide. The concentration of peptides that contain unnatural amino acids absorbing UV light at 280 nm with unknown ε280, or that lack tryptophan or tyrosine, was determined by weighing

2. Stability studies

2.1. Degradation assays in human serum & mouse plasma

To a mixture of 250 µL of human serum (or mouse plasma) and 750 µL of RPMI 1640 were added 20 µL of the peptide DMSO stock solution at 10 mM. The mixture was incubated at 37 °C. Aliquots of 100 µL were removed from the medium at different time, mixed with 100 µL of TCA solution (6%) and incubated at 4 °C for at least 15 min to precipitate all the serum proteins. After centrifugation at 12000 rpm for 2 min, 50 µL of the supernatant were transferred to an injection vial and analyzed by HPLC with a linear gradient of MeCN in water (5 to 95% + 0.1% TFA). The relative concentrations of the remaining soluble peptides were calculated by integration of the absorbance at 220 nm as a function of the retention time (peak area).

2.2. Stability under Chymotrypsin and Trypsin incubation

A 0.6 mL tube was charged with 180 µL of phosphate buffer pH 7.4, 10 µL of enzyme (0.05 mg/mL stock solution in phosphate buffer pH 7.4), 10 µL of peptide (10 mM stock solution in DMSO). The resulting reaction mixture was capped and incubated at room temperature for 3 hours. 20 µL of the crude reaction was quenched by addition of 180 µL of 50% water: 50% acetonitrile and was subjected to LC-MS analysis *Method A* (Figure S1)

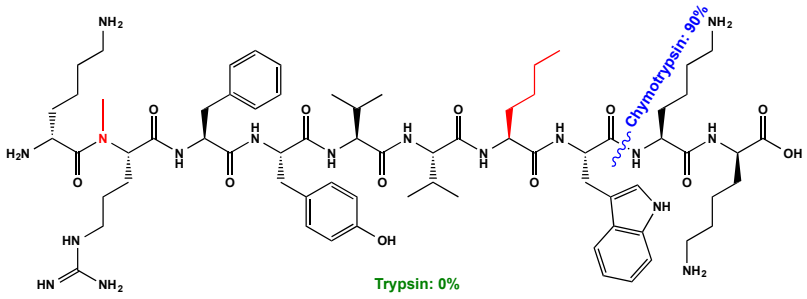


Figure S1. Enzymatic cleavage of PKT16 (peptide **10**)

3. Structural analyses

3.1. CD spectroscopy

CD experiments were acquired on a Jasco J-815 CD spectropolarimeter with a Peltier temperature-controlled cell holder (30 °C) over the wavelength range 190-270 nm. Peptide samples were prepared at a concentration of 50 µM in 10 mM sodium phosphate buffer, pH 7.4, using a quartz cell of 1 mm path length. Measurements were taken every 0.2 nm at a scan rate of 10 nm/min.

3.2. NMR conformational analysis

Lyophilized PKT16 peptide was dissolved at 1 mM concentration in 550 µL of H_2O/D_2O (90:10 v/v). Sodium 4,4-dimethyl-4-silapentane-1-sulfonate-d_6 (DSS, from Sigma Aldrich) was added at a final concentration of 0.11 mM for chemical shift calibration. NMR experiments were recorded on a Bruker Avance III 500 MHz spectrometer equipped with a TCI $^{1}H/^{13}C/^{15}N$

cryoprobe with Z-axis gradient. NMR spectra were processed with TopSpin 3.2 software (Bruker) and analysed with NMRFAM-SPARKY program.[i] ^{1}H, ^{13}C, and ^{15}N resonances were assigned using 1D ^{1}H WATERGATE, 2D ^{1}H-^{1}H TOCSY (DIPSI-2 isotropic scheme of 80 ms duration), 2D ^{1}H-^{1}H ROESY (300 ms mixing time), 2D ^{1}H-^{13}C HSQC, 2D ^{1}H-^{15}N HSQC, and 2D ^{1}H-^{13}C HMBC recorded at 25 °C. ^{1}H chemical shift was referenced against DSS ^{1}H signal and ^{13}C, ^{15}N chemical shifts were referenced indirectly. The chemical shift deviations were calculated as the differences between observed ^{1}H, ^{13}C chemical shifts and random coil values.[ii] $^{3}J_{\mathrm{HN\text{-}H\alpha}}$ coupling constants were measured on 1D ^{1}H WATERGATE experiments recorded at 5 or 25 °C, or on 1D rows extracted from 2D TOCSY acquired with high resolution.

Table S2. ^{1}H, ^{13}C, ^{15}N chemical shifts of PKT16 peptide (500 MHz, 25°C).
Only the assignment of the major form, corresponding to the *trans* isomer of peptide bond 1-2, is indicated.

Residue	^{15}N	^{1}HN	^{13}Cα	^{1}Hα	^{13}CO	Side chain resonances
D-Lys1			54.1	4.41	173.4	^{13}Cβ 32.1; ^{13}Cγ 24.1; ^{13}Cδ 29.3; ^{13}Cε 42.1 ^{1}Hβ 1.86; ^{1}Hγ 1.47; ^{1}Hδ 1.70; ^{1}Hε 3.00
*N*MeArg2		-	60.1	4.84	173.8	^{13}C*N*Me 33.7; ^{13}Cβ 27.4; ^{13}Cγ 27.4; ^{13}Cδ 43.4; ^{13}Cζ 159.7; ^{15}Nε 84.6; ^{1}H*N*Me 2.75; ^{1}Hβ 1.84, 1.66; ^{1}Hγ 1.43; ^{1}Hδ 3.18; ^{1}Hε 7.18
Phe3	122.4	7.99	57.5	4.65	175.1	^{13}Cβ 39.7; ^{13}Cγ 138.9; ^{13}Cδ 131.8; ^{13}Cε 131.5; ^{13}Cζ 130.0; ^{1}Hβ 3.06, 2.92; ^{1}Hδ 7.19; ^{1}Hε 7.34; ^{1}Hζ 7.30
Tyr4	122.9	8.11	57.5	4.58	174.8	^{13}Cβ 39.2; ^{13}Cγ 130.6; ^{13}Cδ 133.4; ^{13}Cε 118.2; ^{13}Cζ 157.2; ^{1}Hβ 2.98, 2.86; ^{1}Hδ 7.06; ^{1}Hε 6.79
Val5	123.2	8.00	62.3	4.00	175.4	^{13}Cβ 33.0; ^{13}Cγ 20.7; ^{13}Cγ′ 21.0; ^{1}Hβ 1.94; ^{1}Hγ 0.90; ^{1}Hγ′ 0.85
Val6	125.1	8.17	62.4	3.97	175.8	^{13}Cβ 32.8; ^{13}Cγ 21.0; ^{13}Cγ′ 21.0; ^{1}Hβ 1.91; ^{1}Hγ 0.89; ^{1}Hγ′ 0.77
Nle7	126.5	8.20	56.6	4.25	176.5	^{13}Cβ 33.5; ^{13}Cγ 29.9; ^{13}Cδ 24.5; ^{13}Cε 16.1;

[i] W. Lee, M. Tonelli and J. L. Markley, NMRFAM-SPARKY: enhanced software for biomolecular NMR spectroscopy, *Bioinformatics*, 2015, **31**, 1325-1327.
[ii] D. S. Wishart, C. G. Bigam, A. Holm, R. S. Hodges and B. D. Sykes, ^{1}H, ^{13}C and ^{15}N random coil NMR chemical shifts of the common amino acids. I. Investigation of nearest-neighbor effects, *J. Biomol. NMR*, 1995, **5**, 67-81.

						^{1}Hβ 1.67, 1.63; ^{1}Hγ 1.25, 1.19; ^{1}Hδ 1.25; ^{1}Hε 0.84
Trp8	122.5	8 .05	57.2	4.66	175.9	^{13}Cβ 29.6; ^{13}Cγ 111.3; ^{13}Cδ1 127.3; ^{13}Cδ2 129.6; ^{13}Cε2 139.1; ^{13}Cε3 121.0; ^{13}Cζ2 114.8; ^{13}Cζ3 122.2; ^{13}Cη2 124.8; ^{15}Nε1 129.6
						^{1}Hβ 3.26; ^{1}Hδ1 7.24; ^{1}Hε1 10.11; ^{1}Hε3 7.61; ^{1}Hζ2 7.49; ^{1}Hζ3 7.14; ^{1}Hη2 7.23
Lys9	123.3	7.97	56.6	4.21	175.3	^{13}Cβ 33.5; ^{13}Cγ 24.5; ^{13}Cδ 29.1; ^{13}Cε 42.2;
						^{1}Hβ 1.70, 1.60; ^{1}Hγ 1.25; ^{1}Hδ 1.60; ^{1}Hε 2.93; ^{1}Hζ 7.52
D-Lys10	124.4	7.60	57.0	4.12	180.1	^{13}Cβ 33.6; ^{13}Cγ 24.7; ^{13}Cδ 29.3; ^{13}Cε 42.0;
						^{1}Hβ 1.76, 1.59; ^{1}Hγ 1.30; ^{1}Hδ 1.61; ^{1}Hε 2.93; ^{1}Hζ 7.50

4. Binding affinity measurements

The binding affinities of peptides for a MEC-1 cancer cells membrane preparation were measured by biolayer interferometry on an Octet RED96 System (Pall FortéBio Corp., Menlo Park, CA). This system monitors interference of light reflected from two sources (an internal reflection surface and the liquid/solid interface of a fiber optic sensor) to measure the rate of binding of molecules to the biosensor surface.

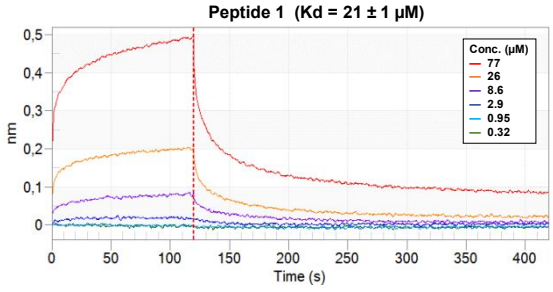
MEC-1 cells membrane preparation is biotinylated with the EZ-Link NHS-PEG4-Biotin kit from Thermo-Scientific and excess biotin is removed using desalting column from Thermo-Scientific. Biotinylated membranes are then loaded onto SuperStreptavidin (SSA) biosensors (Pall FortéBio Corp.) at empirically determined concentrations. All affinity measurements were carried out in assay buffer (PBS with 0.05% Tween 20 and 1% DMSO) at 30 °C.

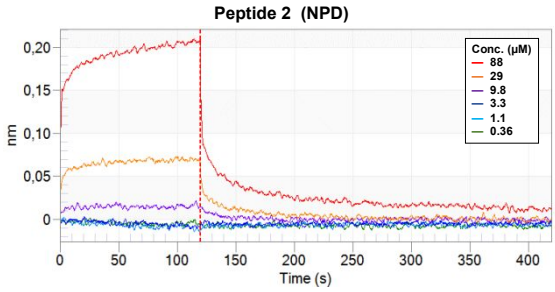
Typically, the biosensors were pre-equilibrated in PBS containing either biotinylated membranes or biocytine 100 µg/mL. Biosensors are then equilibrated in assay buffer for 10min, brought to baseline in assay buffer for 60 sec. and transferred to wells containing peptide in dose-response (association for 120 sec. and dissociation for 300 sec.). The double reference with either membrane-loaded biosensors without any peptide dose-response or biocytine-loaded biosensors with each peptide dose-response were run in parallel for background signal double subtractions. Binding kinetics were calculated using the FortéBio Data Analysis v8.2 software.

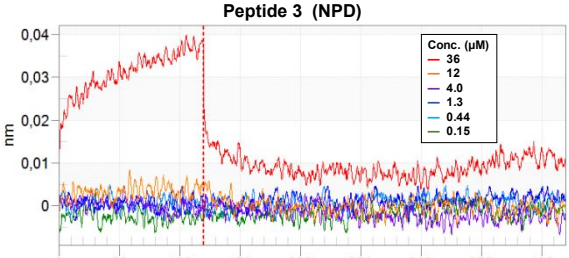
Table S3. Reported apparent Kd values with associated standard deviations (N = 2 ; 3 ; 4 or more). NPD: Non Pertinent Data. ND: Not Determined.

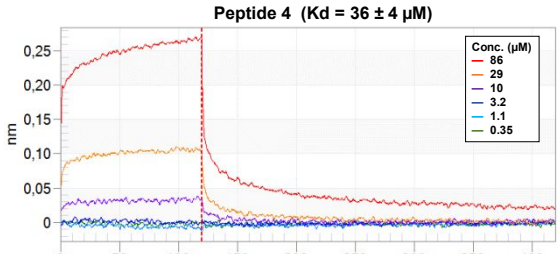
Peptide	Kd
1 (MeR2)	21 ± 1 µM
2 (MeF3)	NPD
3 (MeY4)	NPD
4 (MeV5)	36 ± 4 µM
5 (MeV6)	NPD
6 (MeM7)	NPD
7 (MeW8)	NPD
8 (MeK9)	48 ± 12 µM
9 (MeR2K9)	38 ± 20 µM
10 (PKT16)	8.6 ± 2 µM
11	15 ± 4 µM
12	17 ± 5 µM
13	13 ± 2 µM
14	9.5 ± 3 µM
15	20 ± 4 µM
$[PKHB1]_3$	ND
$[PKT16]_3$	190 ± 90 nM

- *Curves of Binding kinetics observed with Octet Red:*

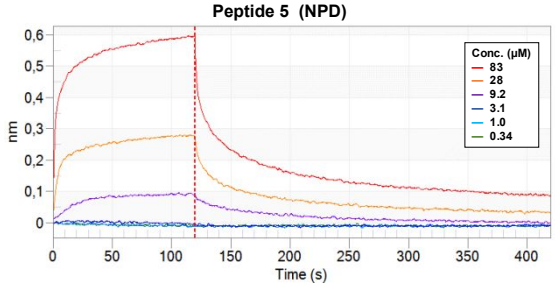




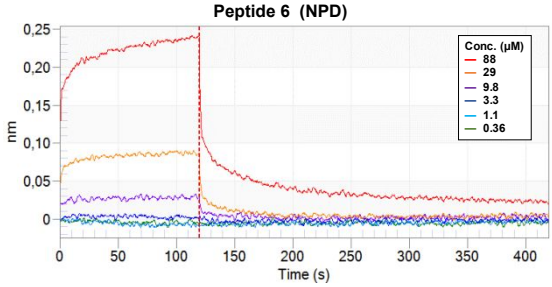




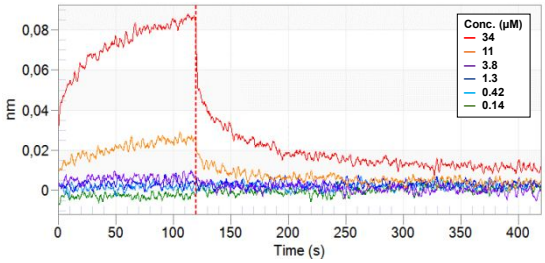
Peptide 5 (NPD)



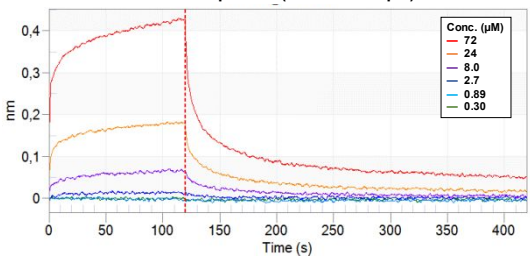
Peptide 6 (NPD)



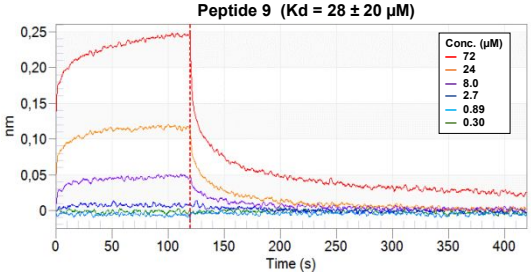
Peptide 7 (NPD)



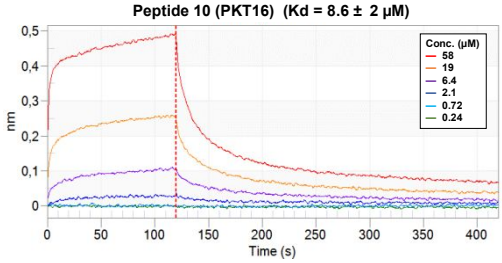
Peptide 8 (Kd = 48 ± 12 µM)



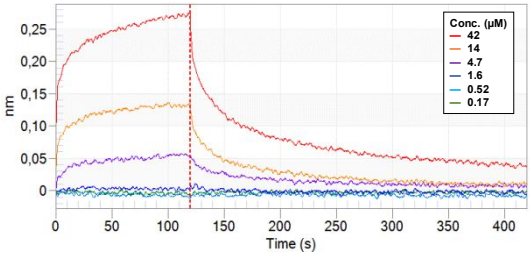
Peptide 9 (Kd = 28 ± 20 µM)



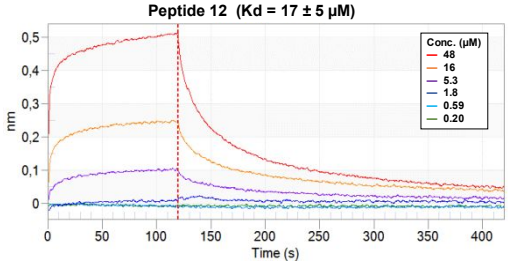
Peptide 10 (PKT16) (Kd = 8.6 ± 2 µM)



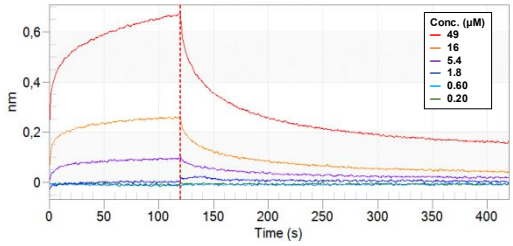
Peptide 11 (Kd = 15 ± 4 µM)



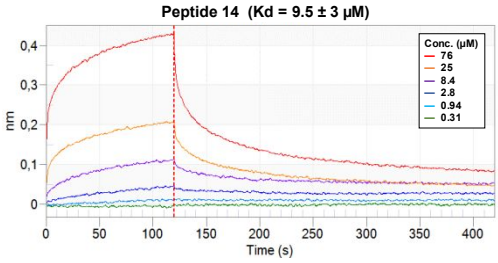
Peptide 12 (Kd = 17 ± 5 µM)



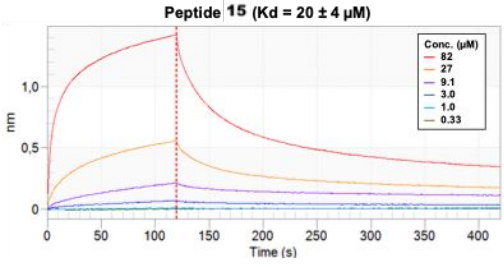
Peptide 13 (Kd = 13 ± 2 µM)



Peptide 14 (Kd = 9.5 ± 3 µM)



Peptide 15 (Kd = 20 ± 4 µM)



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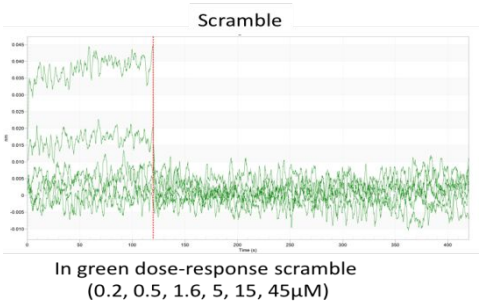
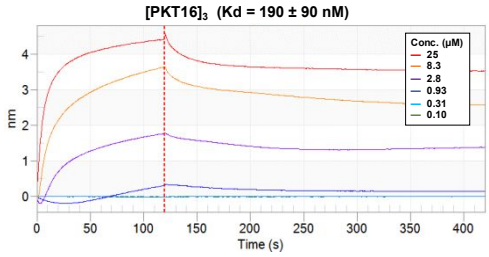
S21





5. Cell culture

Human chronic lymphocytic leukemia cells (MEC-1) and lung adenocarcinoma cells (A549) were grown in Advanced RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS), 100 units/mL penicillin, and 100 µg/mL streptomycin, and maintained following the standard procedures proposed by the American Type Culture Collection.

6. Cell death induction and inhibition

Peptides were solubilized in water or DMSO and stock concentrations were measured by protein absorbance at 280 nm using NanoDrop™ 8000. Only freshly prepared solutions were used for each experiment. Cell death was induced by treating MEC-1 cells for two hours or A549 for six hours with the indicated peptide concentrations. Etoposide (200 µM, 24 h) was used as positive control for p53- and caspase-dependent apoptosis. For the inhibition assays, MEC-1 cells were seeded in non-supplemented medium prior to treatment with inhibitors to limit Ca^{2+} concentration in the medium, while complete medium was used to let A549 cells adhere overnight but was replaced with serum free medium prior to treatment. Calcium chelator, BAPTA (3 mM, CalbioChem; Merck, Billerica, MA, USA) or the pan-caspase inhibitor Q-VD-OPh (10 µM; BioVision, Milpitas, CA, USA) were added 30 minutes before cell death induction, while the PLCγ1 inhibitor U73122 (Sigma-Aldrich), the IP_3R inhibitor 2-APB (Sigma-Aldrich), and the ryanodine receptor inhibitor dantrolene (Sigma-Aldrich) were added one

hour before. Cell death was analyzed in first instance by microscopic observations (Axiovert 40 CFL Zeiss microscope) and comparison of representative photographs (Sony Power HAD 3CCD colour video camera coupled to microscope, 20X). Annexin-V-allophycocyanin (Ann-V-APC 0.1 µg/mL; BD Pharmingen, San Jose CA, USA) and propidium iodide (PI, 0.5 µg/mL; Sigma-Aldrich) in annexin binding buffer (ABB, 10 mM Hepes [pH 7.4], 140 mM NaCl, and 2.5 mM $CaCl_2$) were used to evaluate phosphatidylserine exposure and plasma membrane permeability, respectively. Samples were sorted in a BD FACSCalibur Flow Cytometer (total population 10 000 cells) and data was analysed using FlowJo software (LLC, Ashland, OR, USA). Since peptides-induced cell death consistently presented a double copositive Ann/PI staining, to evaluate cell death in samples where calcium had been chelated, PI staining was used alone in Ca^{2+}[-]/Mg^{2+}[-] DPBS (Gibco) to avoid Ca^{2+} provision by the ABB. For similar reasons, a simpler method as it is trypan blue staining, analysed with an automated counter (Beckman Vi-CELL XR), was used as well to study A549 cell death in the context of PLCγ1-related calcium signalling inhibition. In all cases, the wells were washed twice with Ca^{2+}[-]/Mg^{2+}[-] DPBS (Gibco) after supernatant recovering. A549 cells were generally detached using trypsin-EDTA 0.05% (Gibco), but in some cases chymotrypsin 0.05% (Sigma-Aldrich) was also used to assist $[PKT16]_3$-treated cells detachment. Cell death index was calculated by normalizing peptide-specific cell death (% Cell death - % Cell death in control) in each condition with peptide-specific cell death induced by the peptide alone (Mateo, et al. 2002, doi: https://doi.org/10.1182/blood-2001-12-0217). Two-way ANOVA were performed in GraphPad Prism 8.0 Software for statistical analysis.

Table S4. Effect of caspase and Ca^{2+} signalling inhibition on MEC-1 cell death. MEC-1 cells were pre incubated with vehicle (control), Q-VD-OPH (10 µM), BAPTA (3 mM) U73122 (400 nM), 2-APB (60 µM), dantrolene (80 µM), or a $^1/_3$ combination of the last three (U73122 133 nM, 2-APB 20 µM, dantrolene 27 µM), one hour before treatment with $[PKT16]_3$ (5µM), PKT16 (50µM) or PKHB1 (50µM) in serum free medium. Cell death was analysed by flow cytometry using Ann-V/PI staining and cell death index was obtained by normalizing to 1.00 the cell death induced by each peptide with its corresponding control. The values represent the means (±SD) of at least two independent experiments. §Total cell death induced by etoposide is shown instead of cell death in serum free medium index. *Two-way ANOVA were used to compare problem vs control data populations.

MEC-1	Treatment	Cell death index	SD	Difference from control ($p \leq$)*

	Control	1.00		
	Q-VD-OPH	1.04	0.07	NS
	BAPTA	0.38	0.00	0.0001
$[PKT16]_3$	U73122	0.77	0.03	0.0001
	2-APB	0.69	0.04	0.0001
	Dantrolene	0.75	0.08	0.0001
	D + U + 2	0.42	0.01	0.0001
	Control	1.00		
	Q-VD-OPH	0.95	0.02	NS
	BAPTA	0.00	0.02	0.0001
PKT16	U73122	0.60	0.08	0.0001
	2-APB	0.67	0.01	0.0001
	Dantrolene	0.56	0.14	0.0001
	D + U + 2	0.39	0.05	0.0001
	Control	1.00		
	Q-VD-OPH	0.97	0.02	NS
	BAPTA	0.01	0.07	0.0001
PKHB1	U73122	0.78	0.04	0.001
	2-APB	0.88	0.03	0.05
	Dantrolene	0.79	0.09	0.0001
	D + U + 2	0.64	0.17	0.0001
Etoposide[§]	Control	0.14	0.07	
	Q-VD-OPH	0.07	0.02	NS

Table S5. Effect of caspase and Ca^{2+} signalling inhibition on A549 cell death. A549 cells were pre incubated with vehicle (control), Q-VD-OPH (10 μM), BAPTA (3 mM) U73122 (400 nM), 2-APB (60 μM), dantrolene (80 μM), or a $^1/_3$ combination of the last three (U73122 133 nM, 2-APB 20 μM, dantrolene 27 μM), one hour before treatment with $[PKT16]_3$ (10μM), PKT16 (100μM) or PKHB1 (100μM) in serum free medium. Cell death was analysed by Ann-V/PI and trypan blue staining and cell death index was obtained by normalizing to 1.00 the cell death induced by each peptide with its corresponding control. The values represent the means (±SD) of at least two independent experiments. [§]Total cell death induced by etoposide is shown instead of cell death in serum free medium index. [*]Two-way ANOVA were used to compare problem vs control data populations.

A549	Treatment	Cell death index	SD	Difference from control ($p \leq$)[*]
	Control	1.00		
	Q-VD-OPH	0.99	0.00	NS
	BAPTA	0.52	0.02	0.0001
$[PKT16]_3$	U73122	0.84	0.02	NS
	2-APB	0.77	0.08	NS
	Dantrolene	0.86	0.08	NS
	D + U + 2	0.73	0.04	0.05
	Control	1.00		

PKT16	Q-VD-OPH	1.03	0.14	NS
	BAPTA	0.14	0.14	0.0001
	U73122	0.32	0.15	0.0001
	2-APB	0.72	0.04	0.001
	Dantrolene	0.59	0.23	0.0001
	D + U + 2	0.51	0.22	0.0001
	Control	1.00		
	Q-VD-OPH	1.03	0.01	NS
	BAPTA	0.29	0.06	0.0001
PKHB1	U73122	0.34	0.31	0.0001
	2-APB	0.67	0.09	0.001
	Dantrolene	0.52	0.19	0.0001
	D + U + 2	0.40	0.26	0.0001
Etoposide[§]	Control	0.59	0.03	
	Q-VD-OPH	0.05	0.04	0.0001

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Figure S2. Peptide-induced cell death induces enhanced cell-substrate adhesion: $[PKT16]_3$-treated A549 cells before and after trypsin-EDTA addition.

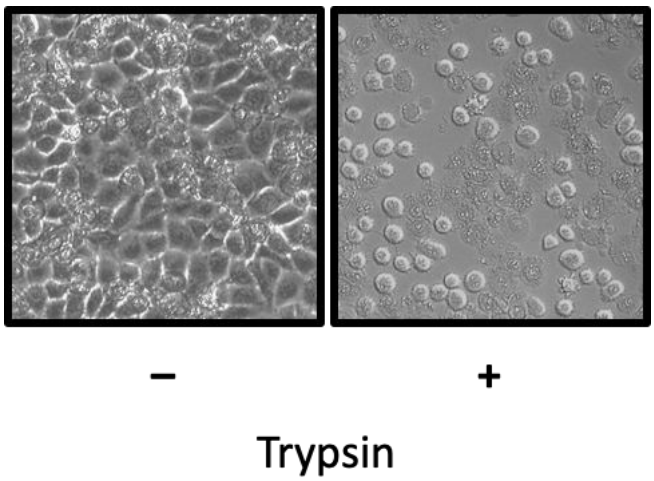
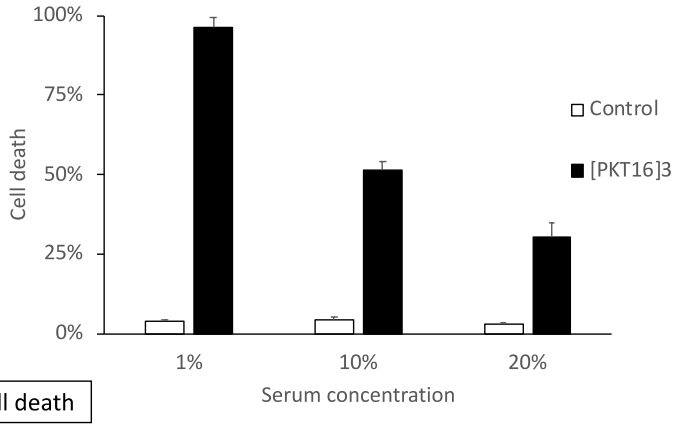


Figure S3. [PKT16]$_3$ activity at different serum concentrations

Treatment\SFB	1%	10%	20%
Control	4%	4%	3%
SD	1%	1%	0%
[PKT16]$_3$	96%	51%	31%
SD	3%	3%	4%



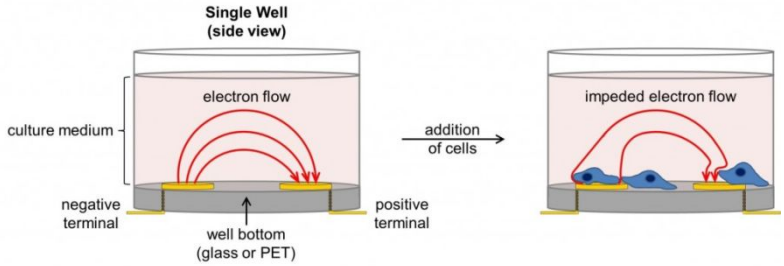
Serum affects peptide's ability to induce cell death

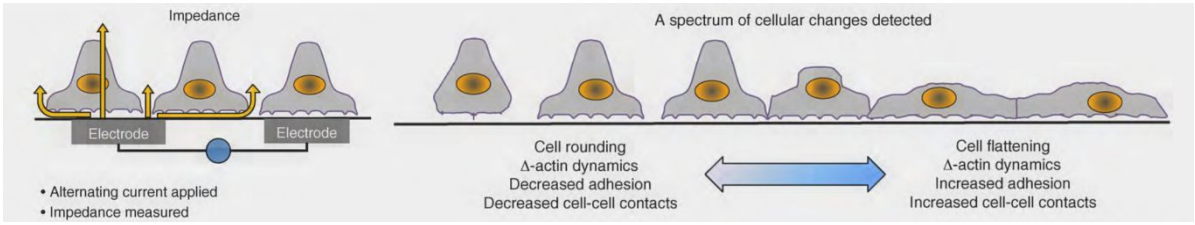
7. Cell viability evaluation through impedance

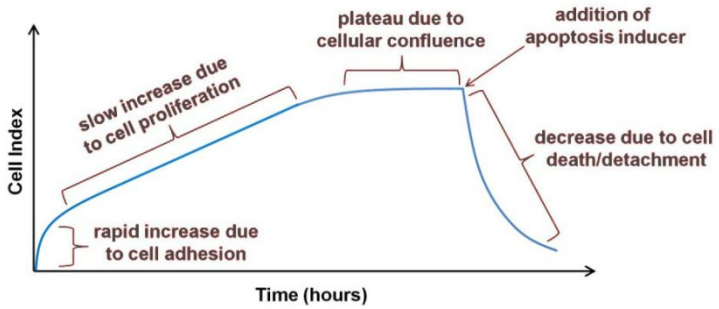
7.1. Brief insights about impedance technology

Label-free detection has emerged as a new approach in the development of technologies for cell-based screening assays. Unlike the classic detection methods that use fluorescence, radioisotope, luminescence, or light absorption, label-free detection directly measures the cell function without using a labeled molecule. The advantages of label-free detection include a simple homogeneous assay format, noninvasive measurement, less interference with normal cell function, kinetic measurement, and reduced time for assay development. Cell-based assays have an important role in drug discovery. Designed appropriately, these *in vitro* tests can help predict the effect of chemical agents *in vivo* and can provide relevant biochemical and pharmacological insight that is not possible in a whole animal study. Label-free technologies with the potential to substantially change some aspects of whole-cell assays have emerged within the past few years. These technologies detect changes in cellular features including adhesion and morphology, complex endpoints that are modulated by many different receptors, ion channels and signal transduction pathway. Currently available label-free instruments use either an impedance-based biosensor or an optical-based biosensor to detect changes in cell behavior.

Here, we have applied the electrical impedance detection method in a real-time cell analyzer (RTCA) system for cell viability assays. The use of impedance to measure cellular processes was first reported by Giaever and Keese at the GE Corporation Research and Development Center. Biosensors measure the impedance to current flow across two electrodes mounted in the base of the microtiter well. Cells that adhere to the surface of the microtiter well restrict the flow of current between the electrodes.





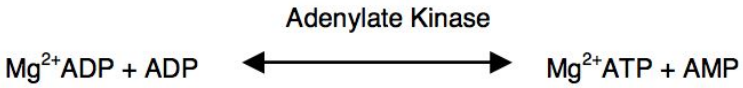


The RTCA monitors cellular events in real time without the incorporation of labels. The system measures electrical impedance across interdigitated microelectrodes integrated on the bottom of tissue culture E-Plates (96-well). The impedance measurement provides quantitative information about the biological status of the cells, including cell number, viability, and morphology. Using the xCELLigence system, we continuously monitored the “Cell

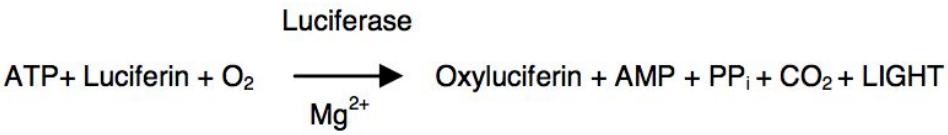
Index" which is derived from the measured impedances and reflects the cell viability under peptide treatment.

7.2. Cytotoxicity assay

The ToxiLight™ bioassay is a non-destructive bioluminescent cytotoxicity assay designed to measure toxicity in mammalian cells and cell lines in culture. The kit quantitatively measures the release of adenylate kinase (AK) from damaged cells. It is a safe, convenient, and highly sensitive method for measuring cytolysis. The assay can be conducted directly cells cultured in a microtiter plate. The kit is based on the bioluminescent measurement of AK which is present in all cells. A loss of cell integrity, through damage to the plasma membrane, results in the leakage of a number of factors from cells cultured in vitro into the surrounding medium. The measurement of the release of AK from the cells allows the accurate and sensitive determination of cytotoxicity and cytolysis. The reaction involves two steps. The first involves the addition of ADP as a substrate for AK. In the presence of the enzyme, AK, the ADP is converted to ATP for assay by bioluminescence:



The bioluminescent method utilizes an enzyme luciferase, which catalyzes the formation of light from ATP and luciferin according to the following reaction:



By combining the two reactions, the emitted light intensity is linearly related to the AK concentration and is measured using a luminometer or beta counter.

Figure S5D. The profound diminution of cell index is correlated to the loss of cell membrane integrity for $[PKT16]_3$. Identification realized by measurement of the release of Adenylate Kinase (AK).

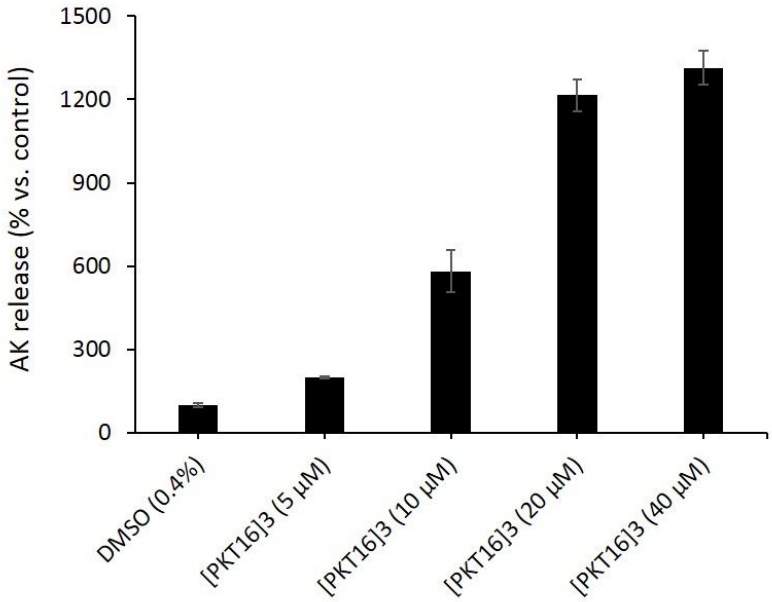
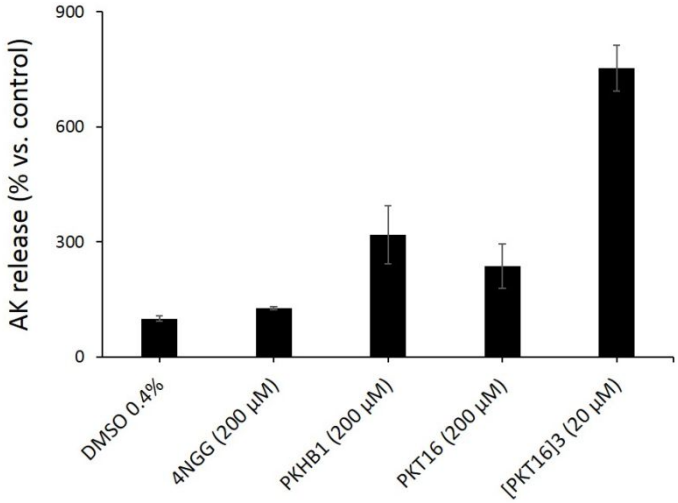


Figure S5E. The profound diminution of cell index is correlated to the loss of cell membrane integrity for PKHB1, PKT16 and $[PKT16]_3$. Identification realized by measurement of the release of AK.



7.3. Protocol for impedance monitoring with our peptides:

The xCelligence RTCA system (Roche) has been described previously. For our experiments, 100 µL of media was added to each well of the 96-well E-plate to measure background levels of impedance. After measuring the background, 100 µL of cell suspension was added to reach a cell density of 4000 cells/well. Cells were allowed to seed at room temperature for 30 min and then placed in the reader at 37 °C and 5% CO_2 for real-time recording of the cell index. The following day, half of the media was removed and replaced with the corresponding volume (100 µL) of media with concentrated peptide in order to reach the desired concentration in the well (direct addition of DMSO caused stress and damaged the cells). Negative controls were treated with the vehicle (DMSO at 0.5% final concentration). Each condition was tested in triplicates and in two independent experiments. The cells were monitored every minute for the first 2 h after treatment and every hour until 24 h after treatment.

9. Molecular formula strings

Compound	SMILES
PKHB1	N[C@H](CCCCN)C(N[C@@H](CCCNC(N)=N)C(N[C@H](C(N[C@H](C(N[C@@H](C(C)C)C(N[C@@H](C(C)C)C(N[C@H](C(N[C@H](C(N[C@@H](CCCCN)C(N[C@H](CCCCN)C(O)=O)=O)=O)CC1=CNC2=C1C=CC=C2)=O)CCSC)=O)=O)=O)CC3=CC=C(O)C=C3)=O)CC4=CC=CC=C4)=O)=O
1	N[C@H](CCCCN)C(N(C)[C@@H](CCCNC(N)=N)C(N[C@H](C(N[C@H](C(N[C@@H](C(C)C)C(N[C@@H](C(C)C)C(N[C@H](C(N[C@H](C(N[C@@H](CCCCN)C(N[C@H](CCCCN)C(O)=O)=O)=O)CC1=CNC2=C1C=CC=C2)=O)CCSC)=O)=O)=O)CC3=CC=C(O)C=C3)=O)CC4=CC=CC=C4)=O)=O
2	N[C@H](CCCCN)C(N[C@@H](CCCNC(N)=N)C(N(C)[C@H](C(N[C@H](C(N[C@@H](C(C)C)C(N[C@@H](C(C)C)C(N[C@H](C(N[C@H](C(N[C@@H](CCCCN)C(N[C@H](CCCCN)C(O)=O)=O)=O)CC1=CNC2=C1C=CC=C2)=O)CCSC)=O)=O)=O)CC3=CC=C(O)C=C3)=O)CC4=CC=CC=C4)=O)=O
3	N[C@H](CCCCN)C(N[C@@H](CCCNC(N)=N)C(N[C@H](C(N(C)[C@H](C(N[C@@H](C(C)C)C(N[C@@H](C(C)C)C(N[C@H](C(N[C@H](C(N[C@@H](CCCCN)C(N[C@H](CCCCN)C(O)=O)=O)=O)CC1=CNC2=C1C=CC=C2)=O)CCSC)=O)=O)=O)CC3=CC=C(O)C=C3)=O)CC4=CC=CC=C4)=O)=O
4	N[C@H](CCCCN)C(N[C@@H](CCCNC(N)=N)C(N[C@H](C(N[C@H](C(N(C)[C@@H](C(C)C)C(N[C@@H](C(C)C)C(N[C@H](C(N[C@H](C(N[C@@H](CCCCN)C(N[C@H](CCCCN)C(O)=O)=O)=O)CC1=CNC2=C1C=CC=C2)=O)CCSC)=O)=O)=O)CC3=CC=C(O)C=C3)=O)CC4=CC=CC=C4)=O)=O
5	N[C@H](CCCCN)C(N[C@@H](CCCNC(N)=N)C(N[C@H](C(N[C@H](C(N[C@@H](C(C)C)C(N(C)[C@@H](C(C)C)C(N[C@H](C(N[C@H](C(N[C@@H](CCCC[NH2]C(C(F)(F)F)=O)C(N[C@H](CCCC[NH2]C(C(F)(F)F)=O)C(O)=O)=O)=O)CC1=CNC2=C1C=CC=C2)=O)CCSC)=O)=O)=O)CC3=CC=C(O)C=C3)=O)CC4=CC=CC=C4)=O)=O
6	N[C@H](CCCCN)C(N[C@@H](CCCNC(N)=N)C(N[C@H](C(N[C@H](C(N[C@@H](C(C)C)C(N[C@@H](C(C)C)C(N(C)[C@H](C(N[C@H](C(N[C@@H](CCCCN)C(N[C@H](CCCCN)C(O)=O)=O)=O)CC1=CNC2=C1C=CC=C2)=O)CCSC)=O)=O)=O)CC3=CC=C(O)C=C3)=O)CC4=CC=CC=C4)=O)=O
7	N[C@H](CCCCN)C(N[C@@H](CCCNC(N)=N)C(N[C@H](C(N[C@H](C(N[C@@H](C(C)C)C(N[C@@H](C(C)C)C(N[C@H](C(N(C)[C@H](C(N[C@@H](CCCCN)C(N[C@H](CCCCN)C(O)=O)=O)=O)CC1=CNC2=C1C=CC=C2)=O)CCSC)=O)=O)=O)CC3=CC=C(O)C=C3)=O)CC4=CC=CC=C4)=O)=O
8	N[C@H](CCCCN)C(N[C@@H](CCCNC(N)=N)C(N[C@H](C(N[C@H](C(N[C@@H](C(C)C)C(N[C@@H](C(C)C)C(N[C@H](C(N[C@H](C(N(C)[C@@H](CCCCN)C(N[C@H](CCCCN)C(O)=O)=O)=O)CC1=CNC2=C1C=CC=C2)=O)CCSC)=O)=O)=O)CC3=CC=C(O)C=C3)=O)CC4=CC=CC=C4)=O)=O
9	N[C@H](CCCCN)C(N(C)[C@@H](CCCNC(N)=N)C(N[C@H](C(N[C@H](C(N[C@@H](C(C)C)C(N[C@@H](C(C)C)C(N[C@H](C(N[C@H](C(N(C)[C@@H](CCCCN)C(N[C@H](CCCCN)C(O)=O)=O)=O)CC1=CNC2=C1C=CC=C2)=O)CCSC)=O)=O)=O)CC3=CC=C(O)C=C3)=O)CC4=CC=CC=C4)=O)=O
Scrambled Peptide	N[C@@H](CCCCN)C(N[C@@H](CC1=CNC2=C1C=CC=C2)C(N[C@H](C(N[C@@H](CCCCN)C(N[C@@H](CC3=CC=C(O)C=C3)C(N[C@@H](CCCNC(N)=N)C(N[C@H](C(N[C@@H](CCSC)C(N[C@@H](CC4=CC=CC=C4)C(N[C@@H](CCCCN)C(O)=O)=O)=O)=O)C(C)C)=O)=O)=O)C(C)C)=O)=O
10 (PKT16)	O=C(N[C@H](CCCCC(O)=O)[C@H](CCCCN)NC([C@H](CC1=CNC2=C1C=CC=C2)NC([C@H](CCCC)NC([C@H](C(C)C)NC([C@H](C(C)C)NC([C@H](CC3=CC=C(O)C=C3)NC([C@H](CC4=CC=CC=C4)NC([C@H](CCCNC(N)=N)N)C(C([C@@H](CCCCN)N)=O)=O)=O)=O)=O)=O)=O
11	N[C@H](CCCCN)C(N(C)[C@@H](CCCNC(N)=N)C(N[C@H](C(N[C@H](C(N[C@@H](C(C)C)C(N[C@@H](C(C)C)C(N[C@H](C(N[C@H](C(N[C@@H](CCCCN)C(N[C@H](CCCCN)C(O)=O)=O)=O)CC1=CNC2=C1C=CC=C2)=O)CC(C)C)=O)=O)=O)CC3=CC=C(O)C=C3)=O)CC4=CC=CC=C4)=O)=O
12	O=C(N(C)[C@@H](CCCNC(N)=N)C(N[C@H](C(N[C@@H](C(C)C)C(N[C@@H](C(C)C)C(N[C@H](C(N[C@H](C(N[C@@H](CCCCN)C(N[C@H](CCCCN)C(N)=O)=O)=O)CC1=CNC2=C1C=CC=C2)=O)[C@H](C)CC)=O)=O)=O)CC3=CC=C(O)C=C3)=O)CC4=CC=CC=C4)=O)[C@@H](CCCCN)NC(C)=O
13	O=C(N(C)[C@@H](CCCNC(N)=N)C(N[C@H](C(N[C@H](C(N[C@@H](C(C)C)C(N[C@@H](C(C)C)C(N[C@H](C(N[C@H](C(N[C@@H](CCCCN)C(N[C@H](CCCCN)C(N)=O)=O)=O)CC1=CNC2=C1C=CC=C2)=O)CC3=CC=C(O)C=C3)=O)=O)=O)CC4=CC=C(O)C=C4)=O)CC5=CC=CC=C5)=O)[C@@H](CCCCN)NC(C)=O
14	O=C(N(C)[C@@H](CCCNC(N)=N)C(N[C@H](C(N[C@H](C(N[C@@H](C(C)C)C(N[C@@H](C(C)C)C(N[C@H](C(N[C@H](C(N[C@@H](CCCCN)C(N[C@H](CCCCN)C(N)=O)=O)=O)CC1=CNC2=C1C=CC=C2)=O)C(C)C)=O)=O)=O)CC3=CC=C(O)C=C3)=O)CC4=CC=CC=C4)=O)[C@@H](CCCCN)NC(C)=O
15	CC(N(C)[C@@H](CCCNC(N)=N)C(N[C@H](C(N[C@H](C(N[C@@H](C(C)C)C(N[C@@H](C(C)C)C(N[C@H](C(N[C@H](C(N[C@@H](CCCCN)C(N)=O)=O)CC1=CNC2=C1C=CC=C2)=O)CCCC)=O)=O)=O)CC3=CC=C(O)C=C3)=O)CC4=CC=CC=C4)=O)=O
$[PKHB1]_3$	O=C(N[C@H](CCCCN)C(O)=O)[C@H](CCCCN)NC([C@H](CC1=CNC2=C1C=CC=C2)NC([C@H](CCSC)NC([C@H](C(C)C)NC([C@H](C(C)C)NC([C@H](CC3=CC=C(O)C=C3)NC([C@H](CC4=CC=CC=C4)NC([C@H](CCCNC(N)=N)NC([C@@H](CCCCN)NC(CCCCN5C=C(CN(CC6=CN(CCCCC(N[C@H](CCCCN)C(N[C@@H](CCCNC(N)=N)C(N[C@@H](CC7=CC=CC=C7)C(N[C@@H](CC8=CC=C(O)C=C8)C(N[C@@H](C(C)C)C(N[C@@H](C(C)C)C(N[C@@H](CCSC)C(N[C@@H](CC9=CNC%10=C9C=CC=C%10)C(N[C@@H](CCCCN)C(N[C@H](CCCCN)C(O)=O)=O)=O)=O)=O)=O)=O)=O)=O)=O)=O)=O)=O)=O)N=N6)CC%11=CN(CCCCC(N[C@H](CCCCN)C(N[C@@H](CCCNC(N)=N)C(N[C@@H](CC%12=CC=CC=C%12)C(N[C@@H](CC%13=CC=C(O)C=C%13)C(N[C@@H](C(C)C)C(N[C@@H](C(C)C)C(N[C@@H](CCSC)C(N[C@@H](CC%14=CNC%15=C%14C=CC=C%15)C(N[C@@H](CCCCN)C(N[C@H](CCCCN)C(O)=O)=O)=O)=O)=O)=O)=O)=O)=O)=O)N=N%11)N=N5)=O)=O)=O)=O)=O)=O)=O
$[PKT16]_3$	O=C(N[C@H](CCCCN)C(O)=O)[C@H](CCCCN)NC([C@H](CC1=CNC2=C1C=CC=C2)NC([C@H](CCCC)NC([C@H](CCSC)NC([C@H](C(C)C)NC([C@H](C(C)C)NC([C@H](CC3=CC=C(O)C=C3)NC([C@H](CC4=CC=CC=C4)NC([C@H](CCCNC(N)=N)N)C)C([C@@H](CCCCN)NC(CCCCN5C=C(CN(CC6=CN(CCCCC(N[C@H](CCCCN)C(N(C)[C@@H](CCCNC(N)=N)C(N[C@@H](CC7=CC=CC=C7)C(N[C@@H](CC8=CC=C(O)C=C8)C(N[C@@H](C(C)C)C(N[C@@H](C(C)C)C(N[C@@H](CCCC)C(N[C@@H](CC9=CNC%10=C9C=CC=C%10)C(N[C@@H](CCCCN)C(N[C@H](CCCCN)C(O)=O)=O)=O)=O)=O)=O)=O)=O)=O)=O)=O)=O)=O)=O)N=N6)CC%11=CN(CCCCC(N[C@H](CCCCN)C(N(C)[C@@H](CCCNC(N)=N)C(N[C@@H](CC%12=CC=CC=C%12)C(N[C@@H](CC%13=CC=C(O)C=C%13)C(N[C@@H](C(C)C)C(N[C@@H](C(C)C)C(N[C@@H](CCCCN)C(N[C@H](CCCCN)C(O)=O)=O)=O)=O)=O)=O)=O)=O)=O)=O)N=N%11)N=N5)=O)=O)=O)=O)=O)=O)=O