Supporting Information

Biophysical and Structural Analyses of the Interaction between the SHANK1 PDZ Domain and an Internal SLiM

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1. Supplementary tables and figures

Table S1. Selection of the tested internal ligands.

Name	Sequence
FAM-Ahx-ARAP3$_{1414-1429}$-CONH$_2$	FAM-Ahx-FPELIQDTSTSFSTTR-CONH$_2$
FAM-Ahx-ELFN1$_{579-594}$-CONH$_2$	FAM-Ahx-ESTSFQGVKSGPVSVA-CONH$_2$
Fluorophore-labelled SLiM	FAM-Ahx-EESTSFQGP-CONH$_2$
SLiM modelled by PSSM	Ac-EESTSFQGP-CONH$_2$

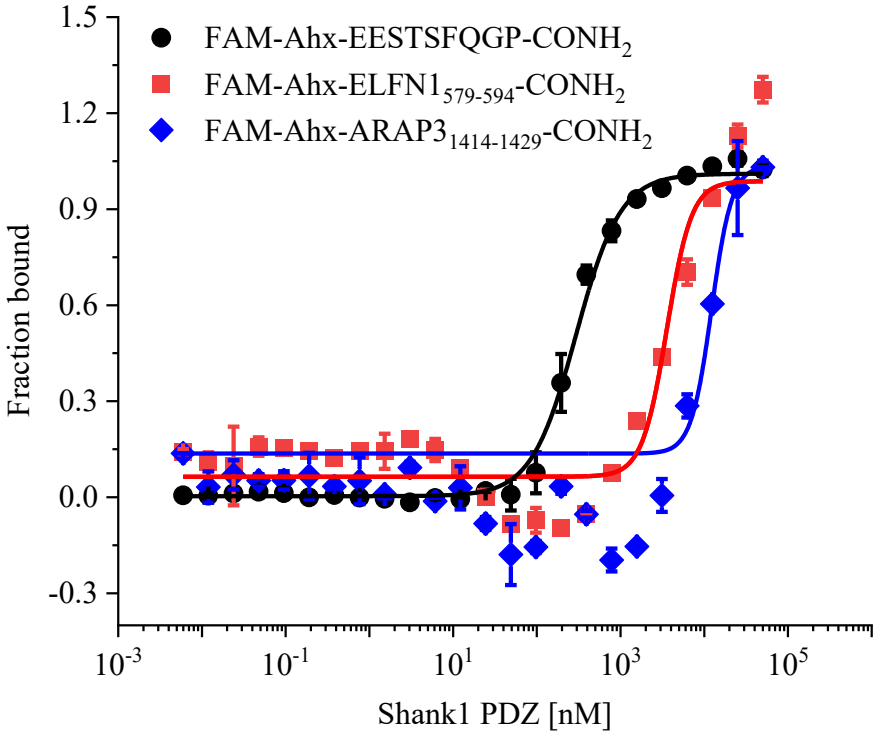


Figure S1. Fluorescence anisotropy direct titration of SHANK1-PDZ in 50 mM NH_4OAc, pH 6.5 buffer using 50 nM FAM-Ahx-ARAP3$_{1414-1429}$-CONH$_2$ (12.2 ± 8.3 µM), FAM-Ahx-ELFN1$_{579-594}$-CONH$_2$ (3.6 ± 0.7 µM), and FAM-Ahx-EESTSFQGP-CONH$_2$ (0.8 ± 0.1 µM) as tracers. Plates was read after 24 hr incubation at room temperature.

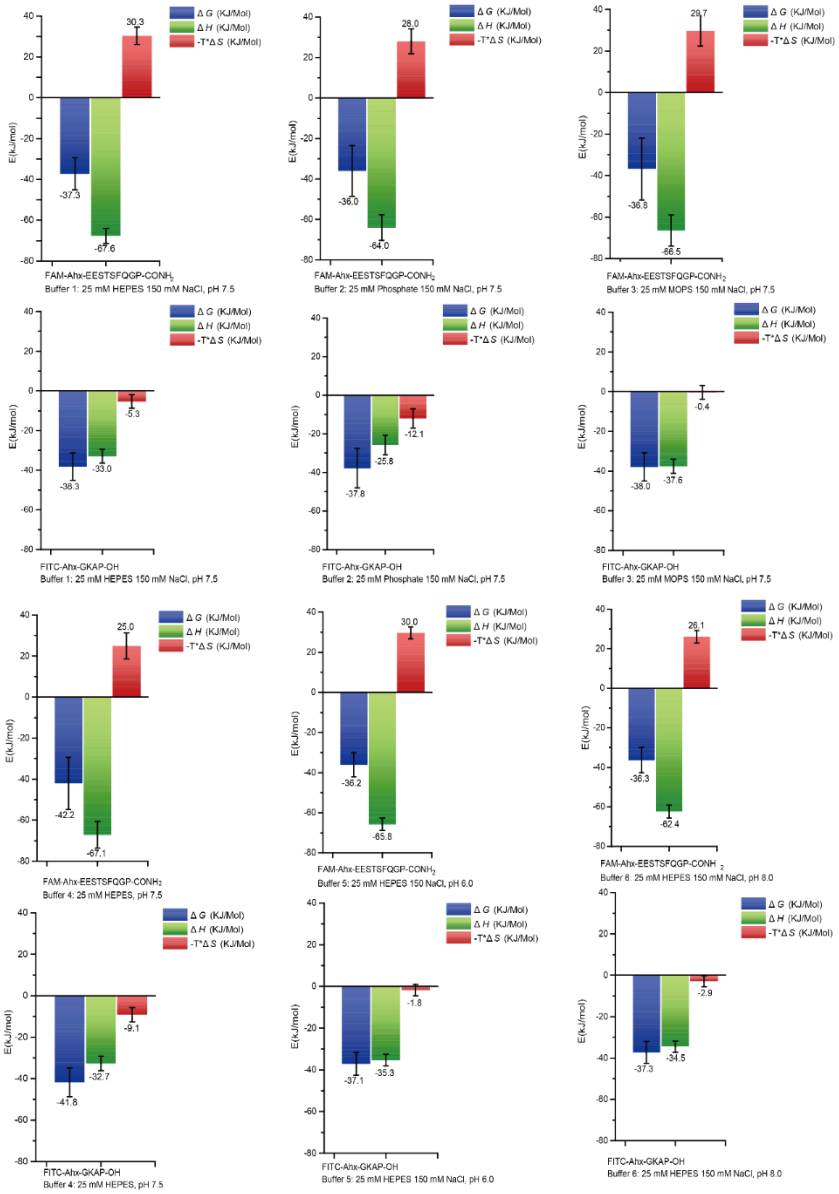


Figure S2. VT-FA assay results of the SHANK1-PDZ with its *N*-terminally fluorophore-labelled internal ligand FAM-Ahx-EESTSFQGP-$CONH_2$ and *C*-terminal ligand FITC-Ahx-EAQTRL-OH. The K_d values were collected from the results of titrating 600 μM SHANK1-PDZ to 50 nM tracers in different buffers (Buffer 1: 25 mM HEPES [4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid], 150 mM NaCl, pH 7.5; Buffer 2: 25 mM phosphate, 150 mM NaCl, pH 7.5; Buffer 3: 25 mM MOPS [3-(N-morpholino)propanesulfonic acid], 150 mM NaCl, pH 7.5; Buffer 4: 25 mM HEPES, pH 7.5; Buffer 5: 25 mM HEPES, 150 mM NaCl, pH 6.0; Buffer 6: 25 mM HEPES, 150 mM NaCl, pH 8.0) at 25 °C, 27.5 °C, 30 °C, 32.5 °C, 35 °C, 37.5 °C and 40 °C. The Δ*G*, Δ*H* and -T*Δ*S* were further calculated using the Arrhenius equation and the Gibbs free energy change equation.

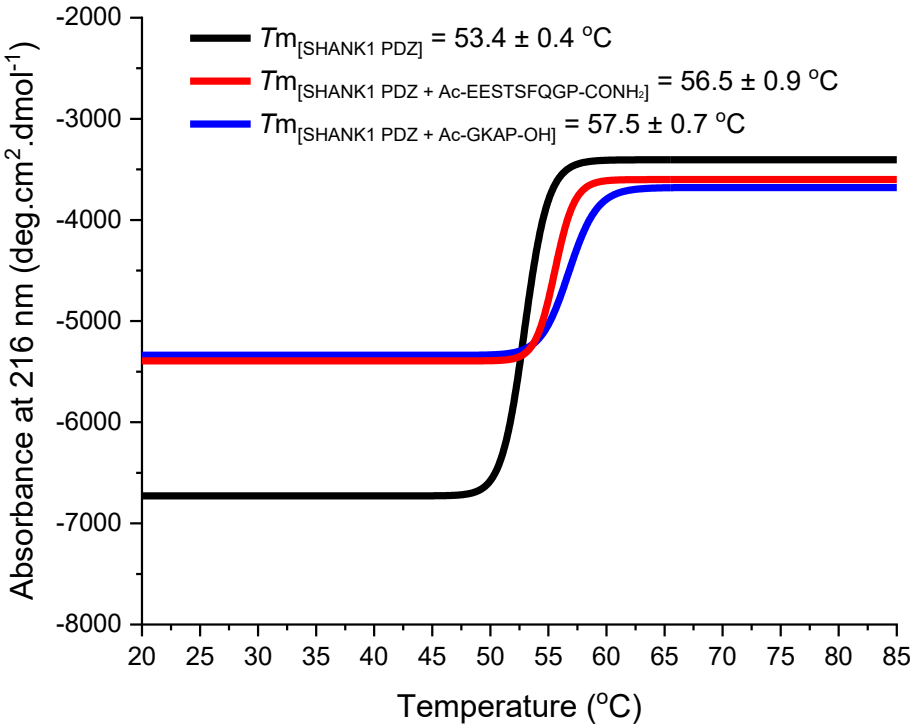


Figure S3. Normalized-fitted temperature melting curves of apo SHANK1-PDZ, SHANK1-PDZ/Internal ligand Ac-EESTSFQGP-$CONH_2$ complex and SHANK1-PDZ/C-terminal ligand Ac-EAQTRL-OH complex.

Table S2. Collection and refinement statistics for obtained SHANK1-PDZ/Ac-EESTSFQGP-$CONH_2$ co-crystal structure

Data Collection Statistics			
X ray source	DLS Beamline i24		
Average unit cell	a, b, c, (Å): 149.10, 149.10, 64.07 α, β, γ (°): 90, 90, 120		
Processed using	Xia2 dials		
/Space group	*P* 3 2 2 1		
	Overall	Inner shell	Outer shell
Low resolution limit	129.16	129.43	2.01
High resolution limit	1.98	5.37	1.98
R_{merge} (all I+ and I-)	0.123	0.061	1.661
R_{meas} (all I+ and I-)	0.129	0.064	1.754
R_{pim} (all I+ and I-)	0.039	0.019	0.560
Total number of obs	1184571	60633	54186
Total number unique	57199	3009	2827
Mean((I)/sd(I))	14.7	51.4	1.2
Completeness	100.0	100.0	100.0
Multiplicity	20.7	20.2	19.2
CC Half	0.999	0.999	0.831
Wilson B Factor ($Å^2$)	34.9		
Model Refinement			
Protein molecules in au	4		
Rwork/Rfree (%)	21.7/23.6		
Number of atoms			
Protein	3321		
Ligand	291		
Water	115		
Mean B factors (Å)			
Protein	50.7		
Ligand	49.2		
Water	42.5		
R.m.s. deviations			
Bond length (Å)	0.01		
Bond angles	1.79		
Ramahandran statistics			
% favoured	98.2		
% allowed	100.0		
% outliers	0		
PDB accession code	8S1R		

Table S3. Observed residual interactions between protomers.

Interactions of protomer I Chain A: promoter II Chain B	**Distance (Å)**
Thr663 main chain NH: Leu653 main chain C=O	3.0
Glu661 main chain NH: Ser655 main chain C=O	2.9
Ile659 main chain NH: Tyr657 main chain C=O	2.9
Ile659 main chain C=O: Tyr657 main chain NH	2.8
Tyr657 main chain C=O: Ile659 main chain NH	3.0
Tyr657 main chain NH: Ile659 main chain C=O	2.9
Ser655 main chain C=O: Glu661 main chain NH	3.0
Leu653 main chain C=O: Thr663 main chain NH	2.9
Interactions of protomer I Chain A: promoter III Chain G	**Distance (Å)**
Glu707 main chain OH: Ser^{-1} side chain OH	3.1
Interactions of protomer III Chain C: promoter IV Chain D	**Distance (Å)**
Arg713 side chain NH: Gln667 main chain C=O	3.2
Gln667 main chain C=O: Ser671 side chain NH	2.9
Met750 side chain CH: Glu707 side chain C=O	3.3
Leu665 main chain NH: Gly708 main chain C=O	2.9

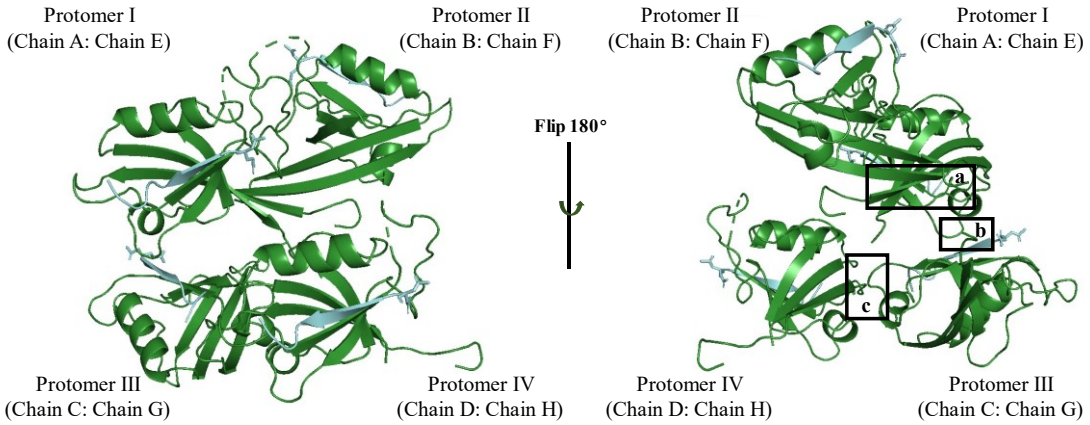


Figure S4. Observed complex of four SHANK1/Ac-EESTSFQGP-NH_2 protomers I, II, III and IV stacking in one asymmetric unit cell.

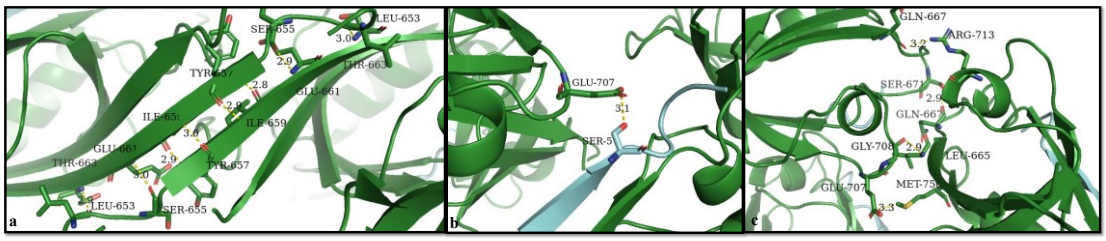


Figure S5. Observed interactions between different protomers: **a.** Antiparallel interaction of β-strands between protomer I and II occurs from residues Leu653^{PDZ}, Ser655^{PDZ}, Tyr657^{PDZ}, Ile659^{PDZ}, Glu661^{PDZ} and Thr663^{PDZ} from Chain A and Chain B (distances of 2.9 – 3.0 Å); **b.** Protomer I interacts with protomer III by forming one hydrogen bond (3.1 Å) between Chain A Glu707^{PDZ} and Chain G Ser(-1); **c.** Protomer III contacts protomer IV by forming four pairs of hydrogen bonds between Chain C residues Arg713^{PDZ}, Gln667^{PDZ}, Met750^{PDZ}, Leu665^{PDZ} and Chain D residues Gln667^{PDZ}, Ser671^{PDZ}, Glu707^{PDZ}, Gly708^{PDZ}.

Table S4. Observed residual interactions between the internal PBM Ac-EESTSFQGP-$CONH_2$ and SHANK1 PDZ domain.

Interactions	**Distance (Å)**			
Ligand-Protein	E-A	F-B	G-C	H-D
Glu^{-5}-$Tyr701^{PDZ}$ (Side chain OH to side chain OH)	2.9	3.0	3.3	2.9
Glu^{-5}-$Arg679^{PDZ}$ (Side chain C=O to side chain NH)	3.0	-	3.2	-
Glu^{-5}-$Arg679^{PDZ}$ (Side chain C=O to side chain NH_2)	3.1	-	-	-
Glu^{-4}-$Gly680^{PDZ}$ (Main chain NH to main chain C=O)	2.9	3.0	3.1	3.3
Glu^{-4}-$Gly680^{PDZ}$ (Main chain C=O to main chain NH)	3.0	3.1	3.1	3.0
Glu^{-4}-$Arg736^{PDZ}$ (Side chain C=O to side chain NH_2)	3.2	3.4	-	-
Glu^{-4}-$Arg736^{PDZ}$ (Side chain OH to side chain NH_2)	3.4	2.8	-	-
Glu^{-4}-$Arg736^{PDZ}$ (Side chain C=O to side chain NH)	-	3.2	2.6	-
Glu^{-4}-$Arg736^{PDZ}$ (Side chain OH to side chain NH)	-	3.3	3.0	-

Ser^{-3}-$Glu703^{PDZ}$ (Side chain OH to side chain C=O)	2.6	2.9	2.6	3.2
Thr^{-2}-$Leu678^{PDZ}$ (Main chain NH to main chain C=O)	2.8	2.8	2.8	3.4
Thr^{-2}-$Leu678^{PDZ}$ (Main chain C=O to main chain NH)	2.9	2.8	3.0	2.9
Thr^{-2}-$His735^{PDZ}$ (Side chain OH to imidazole ring H)	2.5	2.6	2.5	2.8
Ser^{-1}-$Glu707^{PDZ}$ (Side chain OH to main chain OH)	-			
Phe^{0}- $Phe674^{PDZ}$ (Main chain C=O to main chain NH)	3.1	3.0	3.1	3.0
Phe^{0}-$Gly675^{PDZ}$ (Main chain C=O to main chain NH)	2.9	2.8	2.9	3.0
Phe^{0}-$Phe676^{PDZ}$ (Main chain NH to main chain C=O)	2.7	2.7	2.9	2.8
Gln^{1}-$Asp706^{PDZ}$ (Side chain NH to side chain C=O)	3.0	2.9	2.8	3.4

Table S5. RMSD values calculated for different parts of the sequences for the SHANK1/GKAP PBM (grey, PDB code: 1Q3P) and SHANK1/Ac-EESTSFQGP-$CONH_2$ (green, PDB code: 8S1R) structures.

Superimposed sequence	RMSD (Å)
Full length	0.45
αA	0.097
αB	0.135
βA	0.552
βB	0.110
βC	0.125
βD	0.088
βE	0.029
βF	0.292
Loop V705-G709	0.342
Loop Q667-G675	0.311
Loop G745-T748	0.250
Loop N726-G727	0.025
Loop G680-Q700	0.629
Loop N729-G734	0.094
Loop A714-G719	0.150

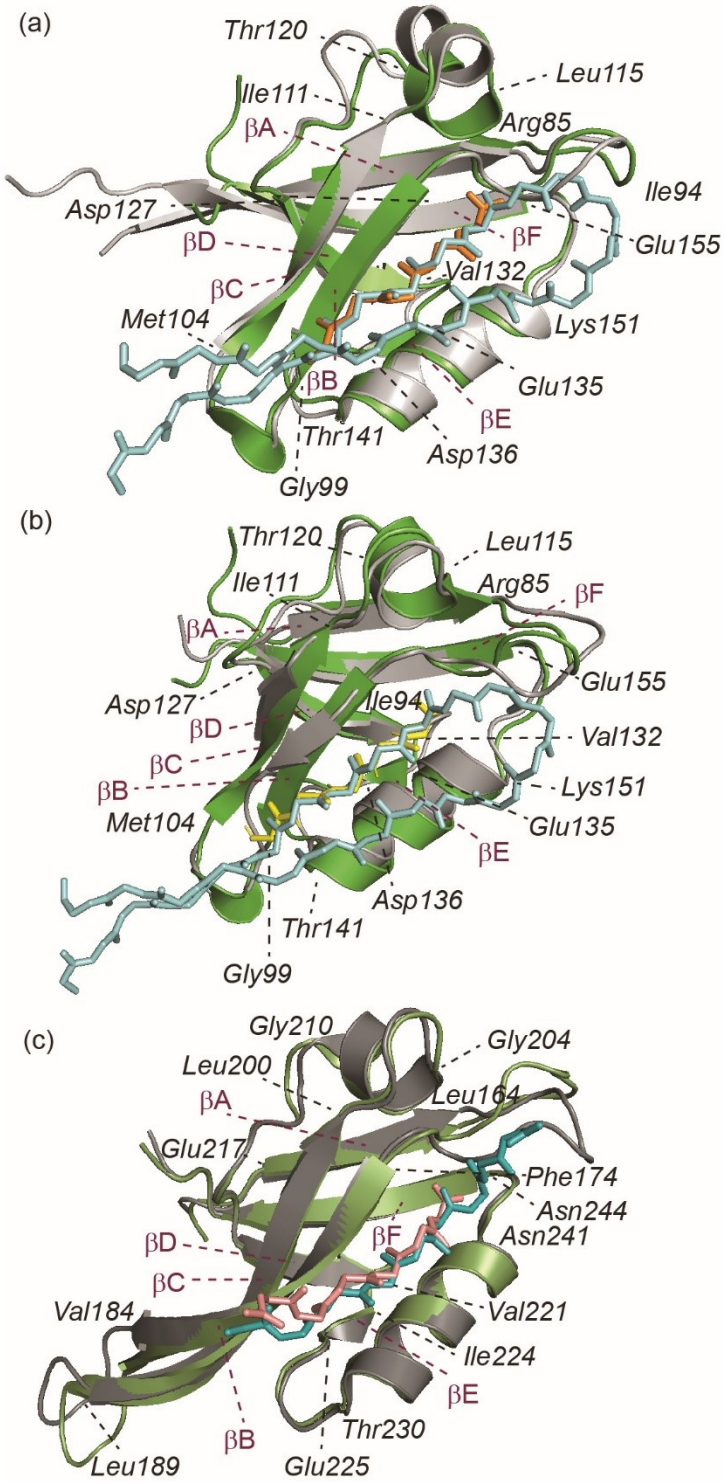


Figure S6. Superimposed crystal structures of (a) Syntrophin PDZ (light grey)/C-terminal PBM (light orange, PDB code: 7QQN) and Syntrophin PDZ (light green)/Internal ligand (light cyan, PDB code: 1QAV) with a full-length calculated RMSD of 0.79 Å; (b) Syntrophin PDZ (light grey)/C-terminal PBM (yellow, PDB code:2PDZ) and Syntrophin PDZ (light green)/Internal ligand (light cyan, PDB code: 1QAV) with a full-length calculated RMSD of 1.153 Å and (c) Par-6 PDZ (dark grey)/C-terminal PBM (salmon orange, PDB code:1X8S) and Par-6 PDZ (smudge green)/Internal ligand (teal cyan, PDB code: 1RZX) with a full-length calculated RMSD of 0.542 Å.

Table S6. RMSD values calculated for different parts of the sequences for the Syntrophin PDZ/C-terminal PBM (PDB code: 7QQN) and Syntrophin PDZ/Internal terminal PBM (PDB code: 1QAV) structures.

Superimposed sequence	RMSD (Å)
Full length	0.786
αA	0.160
αB	0.296
βA	0.163
βB	0.337
βC	0.474
βD	0.160
βE	0.000
βF	0.306
Loop R85-I94	2.365
Loop K151-E155	0.489
Loop V132-E135	0.041
Loop D136-T141	0.002
Loop G99-M104 (including αC)	0.157
Loop I111-L115	0.072
Loop T120-D127	0.183

Table S7. RMSD values calculated for different parts of the sequences for the Syntrophin PDZ/C-terminal PBM (PDB code: 2PDZ) and Syntrophin PDZ/Internal terminal PBM (PDB code: 1QAV) structures.

Superimposed sequence	RMSD (Å)
Full length	1.153
αA	0.156
αB	0.362
βA	0.410
βB	0.352
βC	0.601
βD	0.498
βE	0.016
βF	0.421
Loop R85-I94	1.829
Loop K151-E155	0.378
Loop V132-E135	0.054
Loop D136-T141	0.585
Loop G99-M104 (including αC)	0.314
Loop I111-F112	0.427
Loop T120-D127	0.768

Table S8. RMSD values calculated for different parts of the sequences for the Par-6 PDZ/C-terminal PBM (PDB code: 1X8S) and Syntrophin PDZ/Internal terminal PBM (PDB code: 1RZX) structures.

Superimposed sequence	RMSD (Å)
Full length	0.542
αA	0.271
αB	0.281
βA	0.478
βB	0.505
βC	0.443
βD	0.082
βE	0.001
βF	0.210
Loop L164-F174	2.032
Loop N241-N244	0.129
Loop V221-I224	0.072
Loop E225-T230	0.098
Loop V184-L189	0.378
Loop L200-G204	0.291
Loop G210-E217	0.190

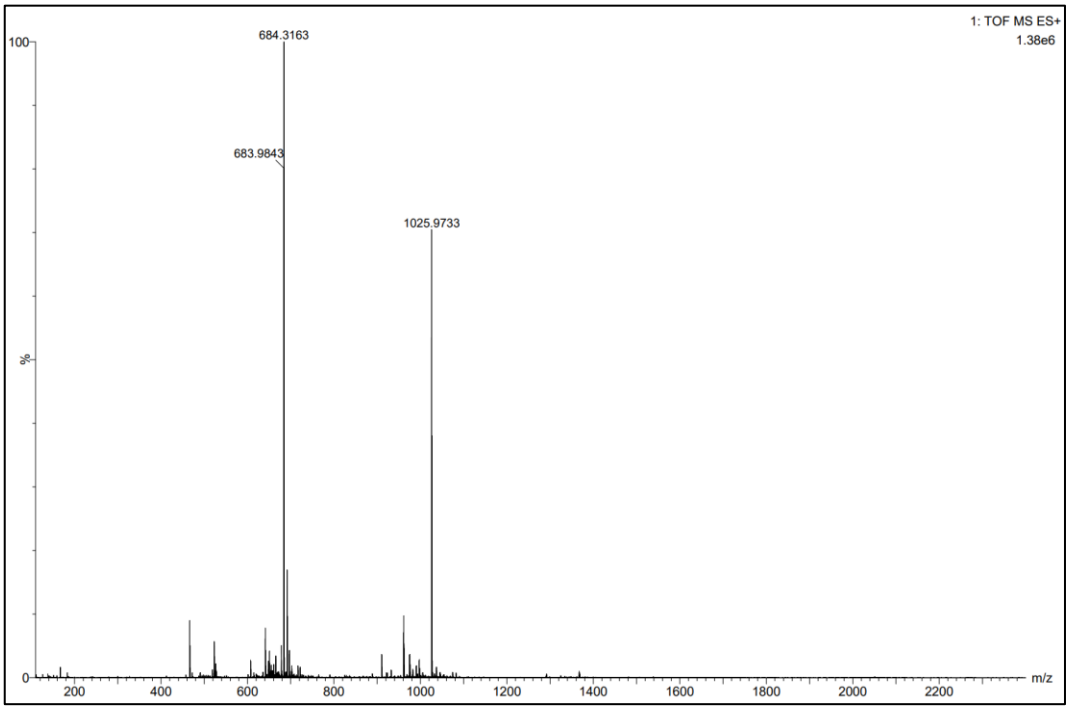
2. Characterisation data

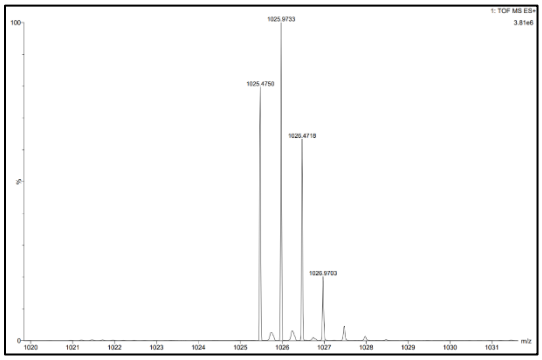
2.1 Mass spectra data of the tested peptides

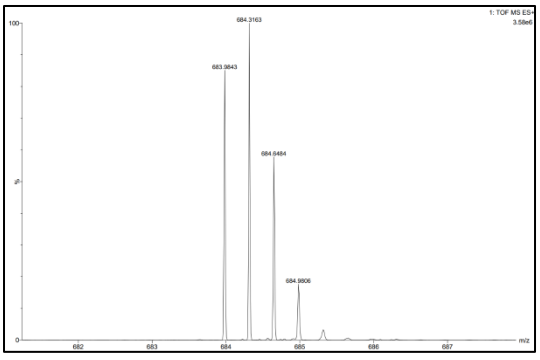
FAM-Ahx-ELFN1$_{579-594}$

Exact Mass: 2048.9367

Expected $[M+2H^+]^{2+}$	Measured $[M+2H^+]^{2+}$
1025.4764	1025.4750
Expected $[M+3H^+]^{3+}$	Measured $[M+3H^+]^{3+}$
683.9869	683.9843



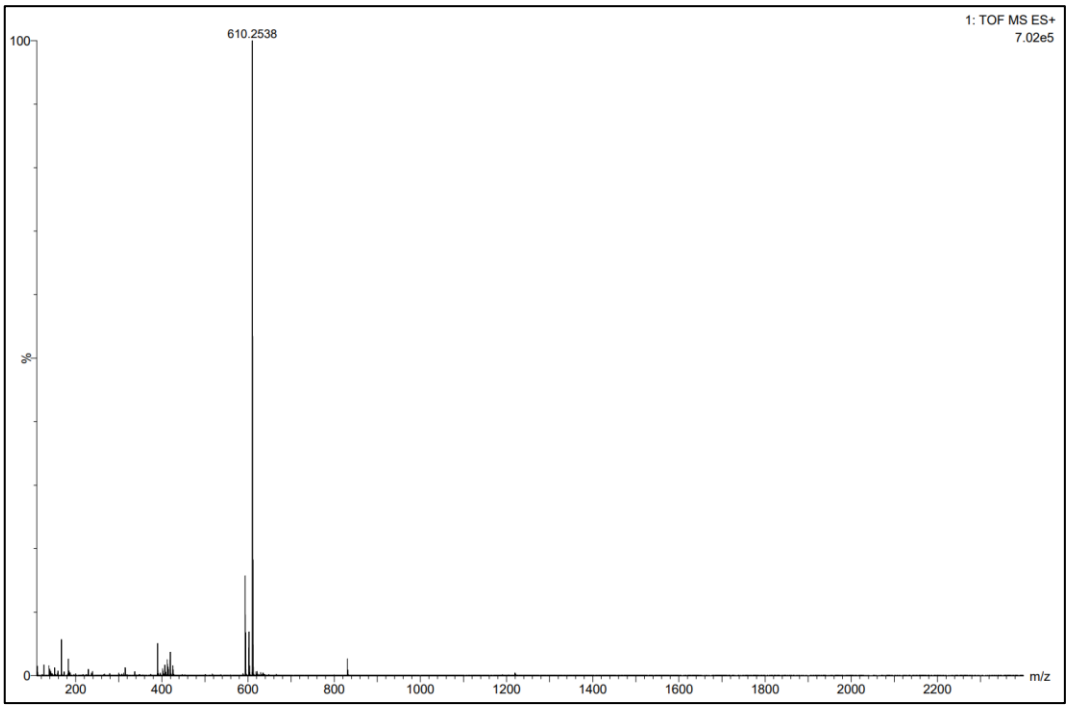


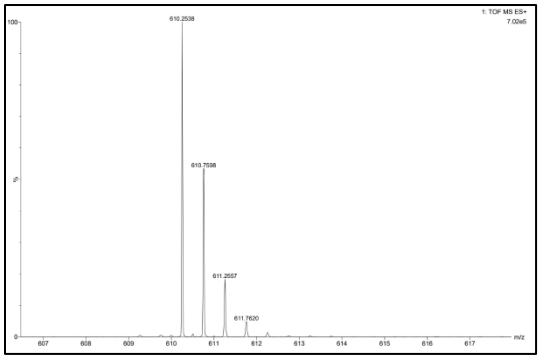


FITC-Ahx-GKAP-OH

Exact Mass: 1218.5016

Expected $[M+2H^+]^{2+}$	Measured $[M+2H^+]^{2+}$
610.2588	610.2538

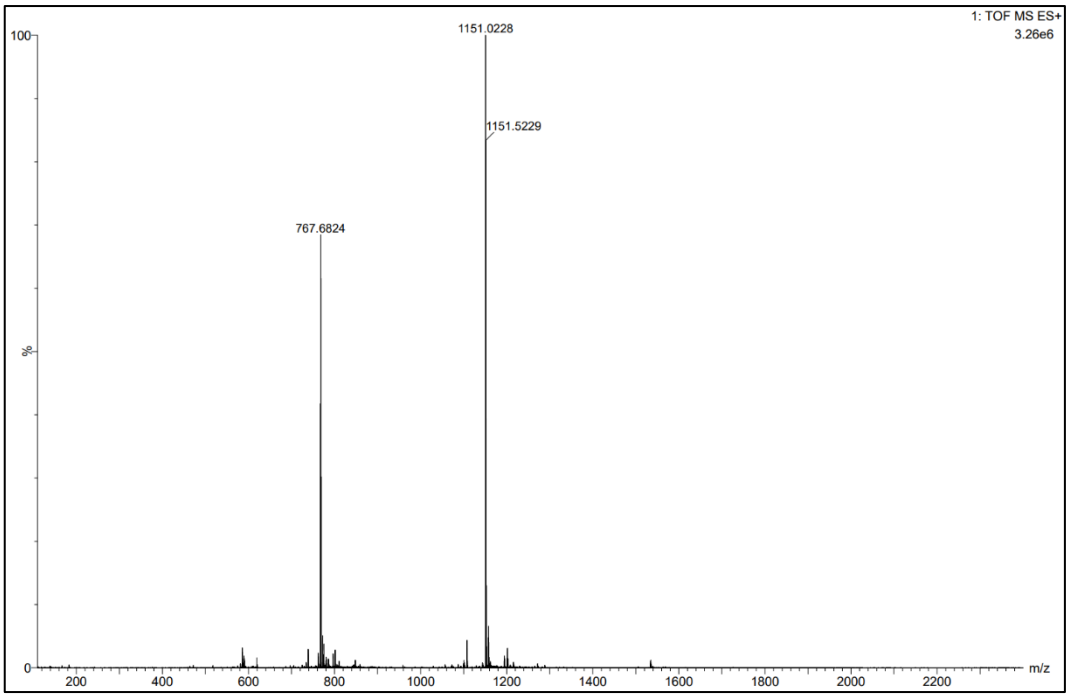


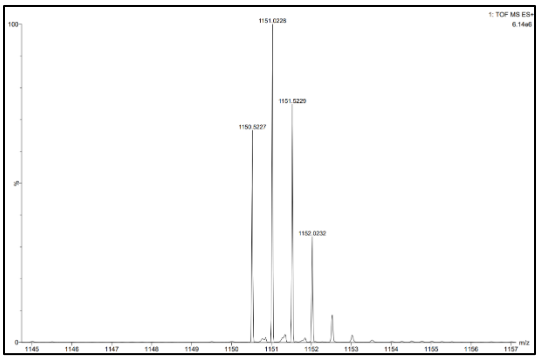


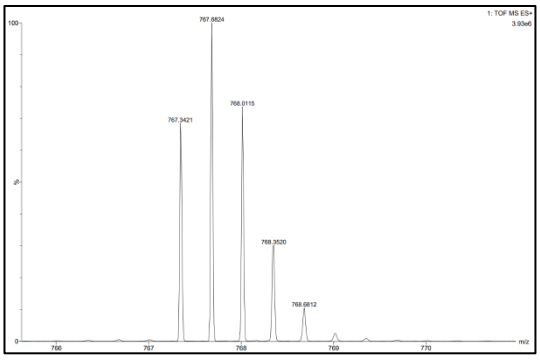
FAM-Ahx-ARAP3$_{1414-1429}$

Exact Mass: 2299.0321

Expected $[M+2H^+]^{2+}$	Measured $[M+2H^+]^{2+}$
1150.5241	1150.5227
Expected $[M+3H^+]^{3+}$	Measured $[M+3H^+]^{3+}$
767.3520	767.3421



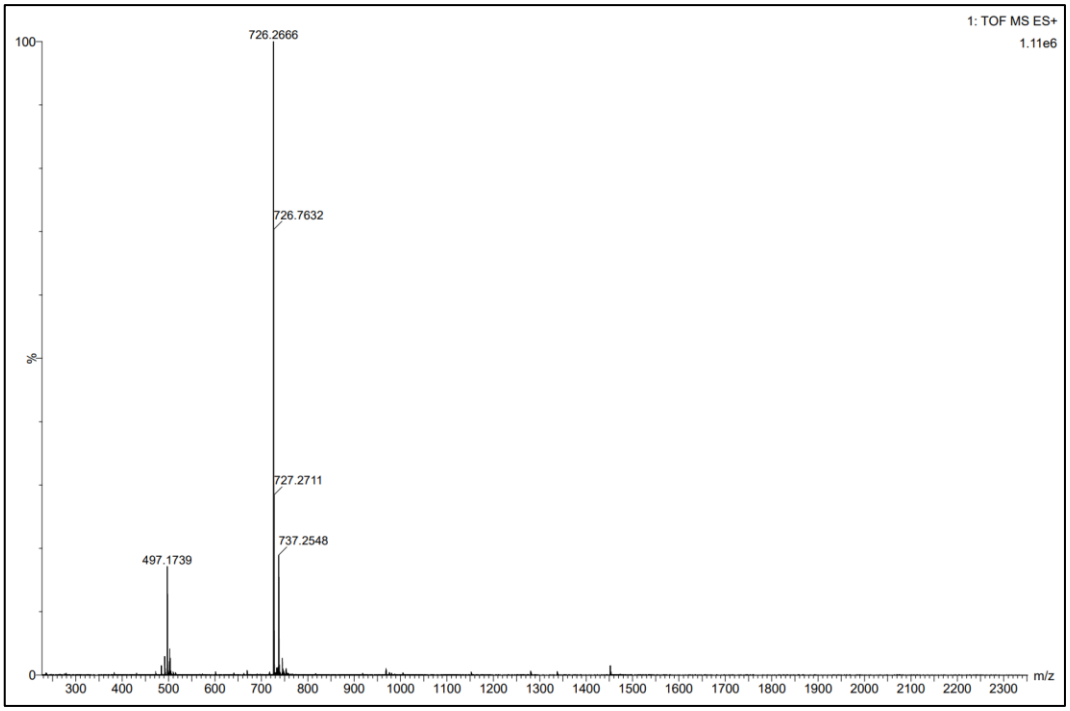


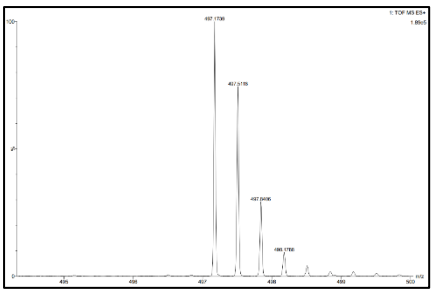


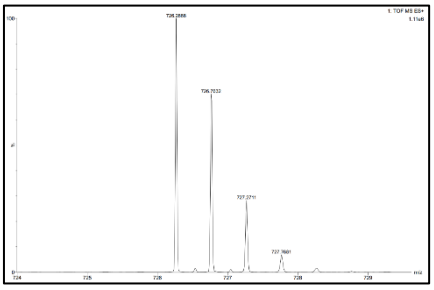
FAM-Ahx-EESTSFQGFP-CONH$_2$

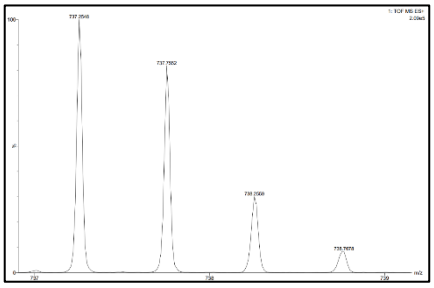
Exact Mass: 1450.5565

Expected $[M+2H^+]^{2+}$	Measured $[M+2H^+]^{2+}$
726.2783	726.2666
Expected $[M+H^++Na^+]^{2+}$	Measured $[M+H^++Na^+]^{2+}$
737.2668	737.2548
Expected $[M+2H^++K^+]^{3+}$	Measured $[M+2H^++K^+]^{2+}$
497.1784	497.1739





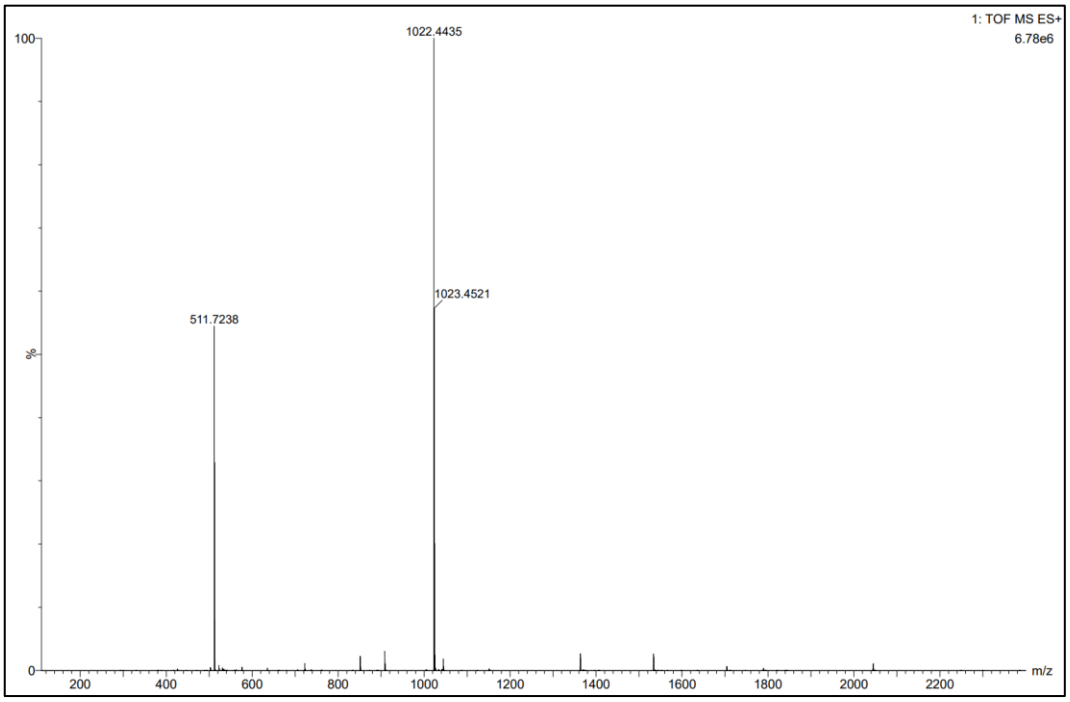


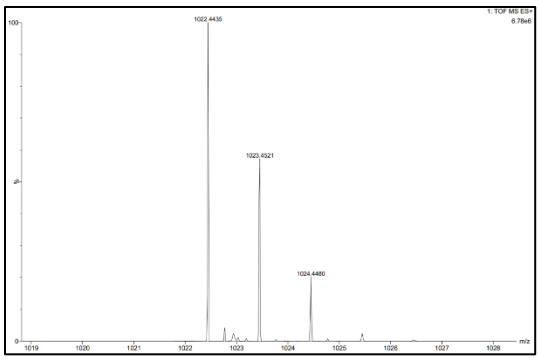


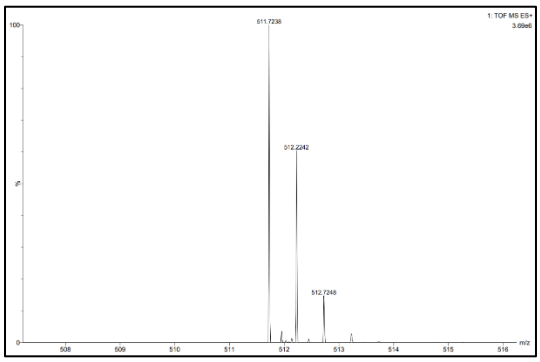
Ac-EESTSFQGP-CONH$_2$

Exact Mass:1021.4353

Expected $[M+H^+]^+$	Measured $[M+H^+]^+$
1022.4425	1022.4435
Expected $[M+2H^+]^{2+}$	Measured $[M+2H^+]^{2+}$
511.7257	511.7238



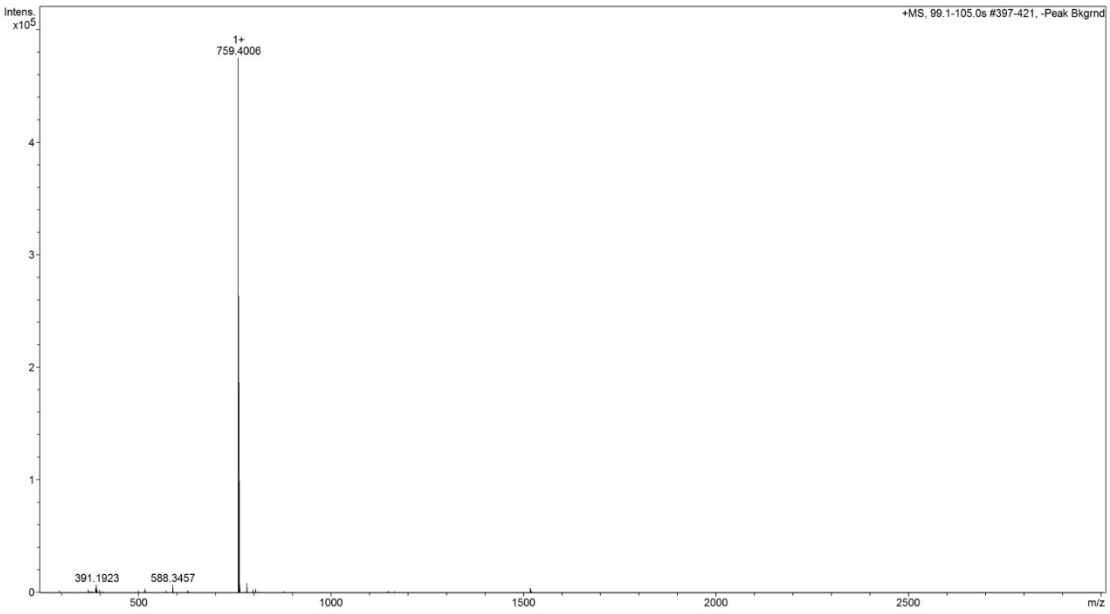


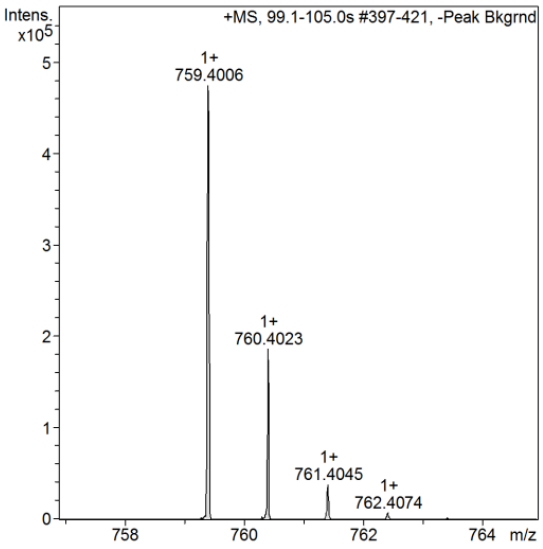


Ac-EAQTRL-CONH$_2$

Exact Mass:758.3923

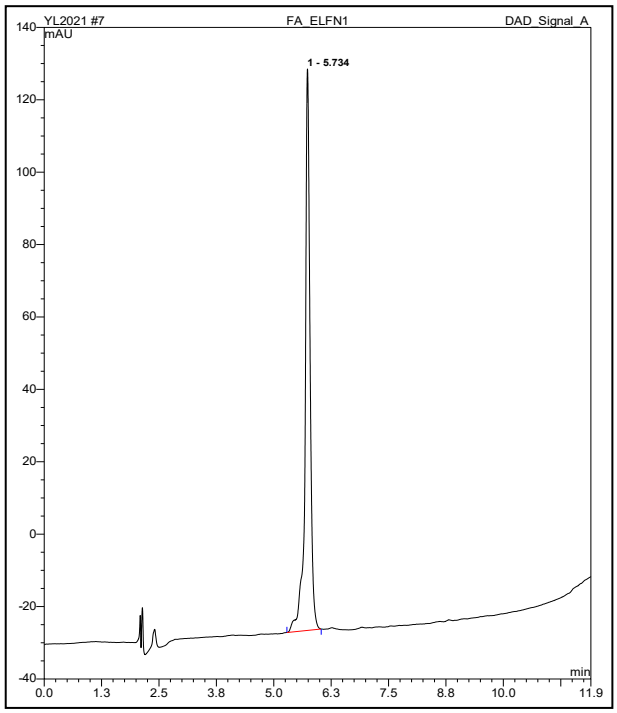
Expected $[M+H^+]^+$	Measured $[M+H^+]^+$
759.3995	759.4006



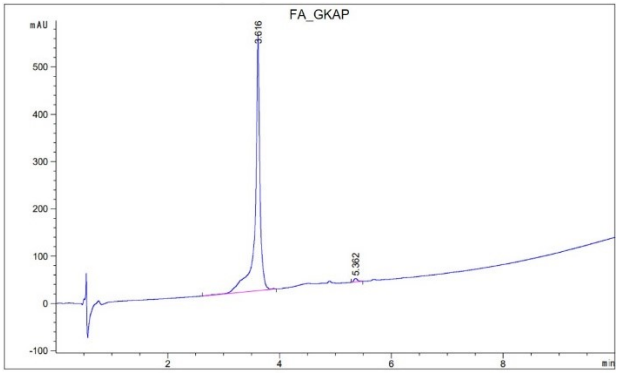


2.2 HPLC analytical purity data of the tested peptides

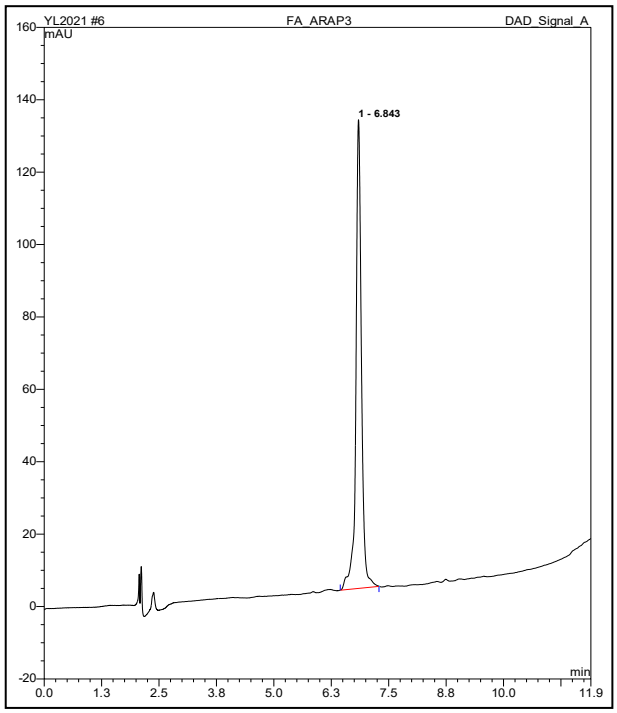
FAM-Ahx-ELFN1$_{579-594}$



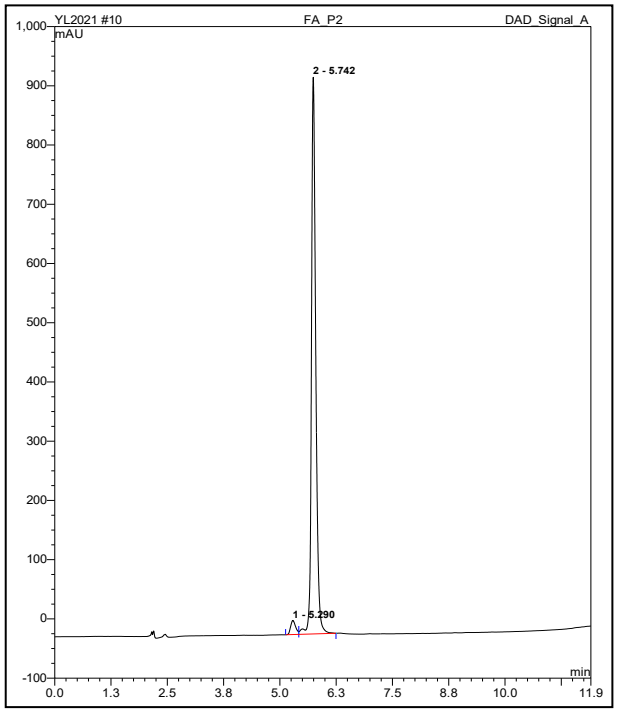
FAM-Ahx-GKAP-OH



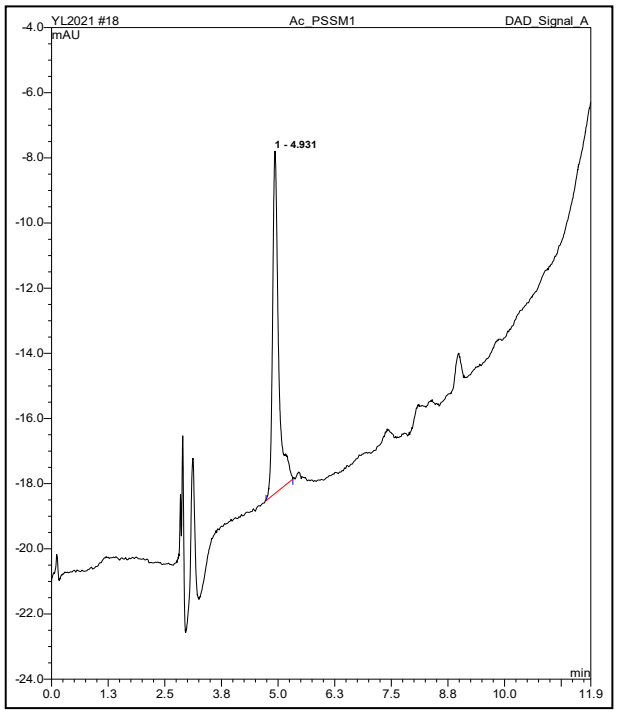
FAM-Ahx-ARAP3$_{1414-1429}$



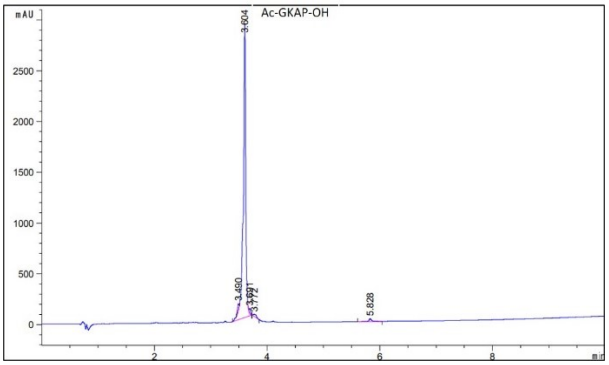
FAM-Ahx-EESTSFQGP-CONH$_2$



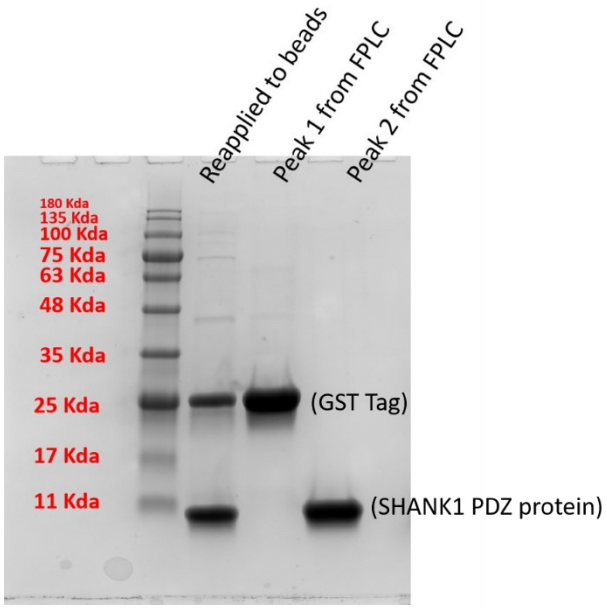
Ac-EESTSFQGP-CONH$_2$



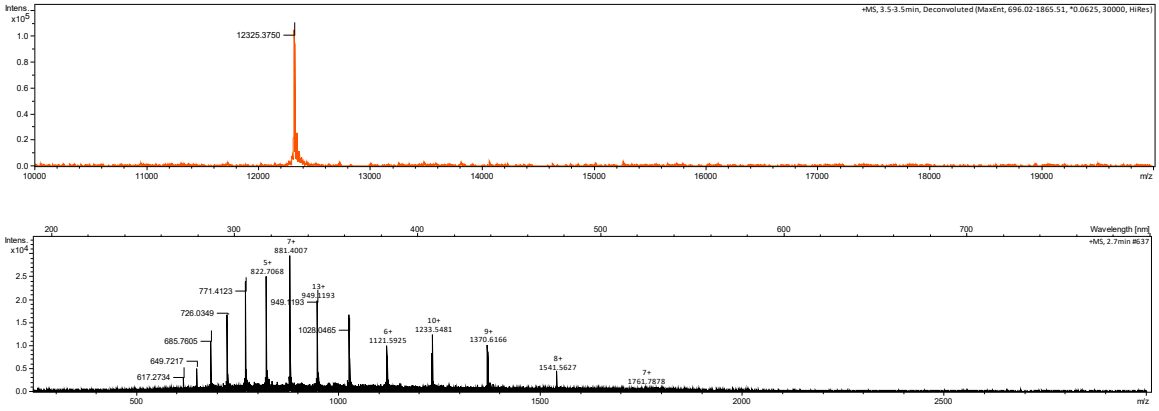
Ac-EAQTRL-CONH$_2$



2.3 SDS-PAGE gel for the SHANK1 PDZ protein expression and purification



2.4 Mass spectrum of the SHANK1 PDZ protein expression



2.5 Chromatographic conditions for preparative HPLC[α]

Time (min)	Mobile phase A (%)	Mobile phase B (%)	Flow (mL/min)
0	90	10	15
20	50	40	15

Time (min)	Mobile phase A (%)	Mobile phase B (%)	Flow (mL/min)
25	20	80	15
30	0	100	15
33	0	100	15
35	90	10	15

[α]. Reversed Phase C18 column, mobile phase A (0.1% TFA in water), mobile phase B (0.1% TFA in ACN).

2.6 Equations for data calculation in fluorescent anisotropy direct titration

The intensity and anisotropy were calculated by using Equations 1 and 2. The data was fitted to a sigmoidal logistic model to obtain r_{min} and r_{max}, and the L_b was further calculated (Equation 3). Finally, the data was fitted to a nonlinear curve model (Equation 4), to obtain the value of K_D.

$$I = (2PG) + S \qquad \textit{Equation 1}$$

$$r = (S-PG)/I \qquad \textit{Equation 2}$$

$$L_b = (r - r_{min})/[\lambda(r_{max}-r) + (r-r_{min})] \qquad \textit{Equation 3}$$

$$y = \{(k+x+[FL]) - \sqrt{\{k+x+[FL]^2-4*[FL]\}}\}/2 \qquad \textit{Equation 4}$$

I = total intensity, r = anisotropy, P = perpendicular intensity, S = parallel intensity, G is an instrument gain factor, L_b = fraction ligand bound, $\lambda = I_{bound}/I_{unbound} = 1$, [FL] = concentration of fluorescent ligand, $k = K_D$ and x = [added titrant].

2.7 Equations for data calculation in fluorescent anisotropy competition assays

The calculated average anisotropy values and their standard deviation were fitted using a sigmoidal logistic model (Equation 5).

$$y = r_{max} + (r_{min}-r_{max})/(1+(x/x_0)^p) \qquad \textit{Equation 5}$$

y = r = anisotropy, x_o = mid-point of the curve between the r_{max} and r_{min} plateau.