SUPPORTING INFORMATION

Cyclic peptides incorporating phosphotyrosine mimetics as potent and specific inhibitors of the Grb7 breast cancer target.

Gabrielle M. Watson, Menachem J. Gunzburg, Nigus D. Ambaye, William A.H. Lucas, Daouda A. Traore, Ketav Kulkarni, Katie M. Cergol, Richard J. Payne, Santosh Panjikar, Stephanie C. Pero, Patrick Perlmutter, Matthew C.J. Wilce and Jacqueline A. Wilce.

Contents of Supporting Information

I. Supplementary Materials and Methods

GST-Grb7 Full-length expression and purification

The pGex6p2 plasmid containing the Grb7 full-length insert (encoding residues 1-532) was expressed as a GST fusion protein in *Escherichia coli* strain BL21(DE3)pLysS and purified using glutathione affinity chromatography similarly to the Grb7-SH2 domain as described previously [1,2]. Notable exceptions are the inclusion of a complete protease inhibitor tablet in the lysis buffer. Following lysis by sonication, Triton X-100 concentration was increased to 1% and stirred for 1 h at 4 °C prior to centrifugation for 45 minutes at 20,000 g. The cleared lysate was immobilized on a GS Trap FF (GE Healthcare) at 4 °C, washed with buffer containing PBST (1xPBS, 0.5% Triton X-100) and 1mM DTT), then 2M NaCl and lastly with PBST and 1 mM DTT again. The protein was eluted with PBST buffer containing 10 mM reduced glutathione and 1 mM DTT. The eluted protein was further purified by size-exclusion chromatography as described previously for the Grb7 SH2 domain. The final concentration of GST-Grb7-Full-length was determined using a Bradford protein assay. [3]

Binding studies to GST-Grb7 Full-length using surface plasmon resonance

Experiments were conducted on a BIAcore T100 using BIAcore CM5 series S sensor chips as previously reported [4]. The immobilization buffer consisted of 50 mM $NaPO_4$, 150 mM NaCl and 1mM DTT (pH 7.4). Polyclonal anti-GST antibody was immobilized on the reference and active flow cells using amine coupling of the antibody to the surface of the chip (GE Healthcare GST capture kit). For this, the sensor chip was activated with 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride and N-hydroxysuccinimide, then polyclonal anti-GST antibody (at 60 μg/ mL) in 10 mM sodium acetate pH 5.0 injected over the chip surface at 5 μL/ min for 7 min. The flow cells were then blocked with 1 M ethanolamine, resulting in anti-GST antibody immobilization levels between 4004 RU and 6593 RU.

GST alone was immobilized on the reference flow cell and the GST-fusion proteins of Grb7-Full-length and Grb7-SH2 immobilized on the active flow cells so that binding in the three flow cells could be simultaneously assessed. GST, Grb7-Full-length and GST-Grb7-SH2 (all at 0.7 μM in immobilization buffer) were injected over the corresponding flow cells for 7 min at 5 μL/ min with 634 RU, 2256 RU and 11265 RU immobilized, respectively. Grb7-Full-length and GST-Grb7-SH2 (2 μM) were re-injected over the corresponding flow cells for 7 min at 5 μL/ min to reach total RU of 2668 RU and 1463 RU, respectively. Triplicate or duplicate samples of peptide were injected for 60 s at 30 μL/ min, with 300 s dissociation in a buffer containing 1 mM $NaPO_4$, 20 mM Tris, 150 mM NaCl and 1 mM DTT (pH 7.4). The data were analyzed using Scrubber2.0 (BioLogic Software, Campbell, ACT, Australia) and SigmaPlot version 12.0 (Systat Software, Inc, Chicago, IL, USA) to determine K_D values .

REFERENCES

1. Gunzburg, M. J.; Ambaye, N. D.; Del Borgo, M. P.; Pero, S. C.; Krag, D. N.; Wilce, M. C.; Wilce, J. A. Interaction of the non-phosphorylated peptide G7-18NATE with Grb7-SH2 domain requires phosphate for enhanced affinity and specificity. *J. Mol. Recognit.* **2012,** *25*, 57-67.

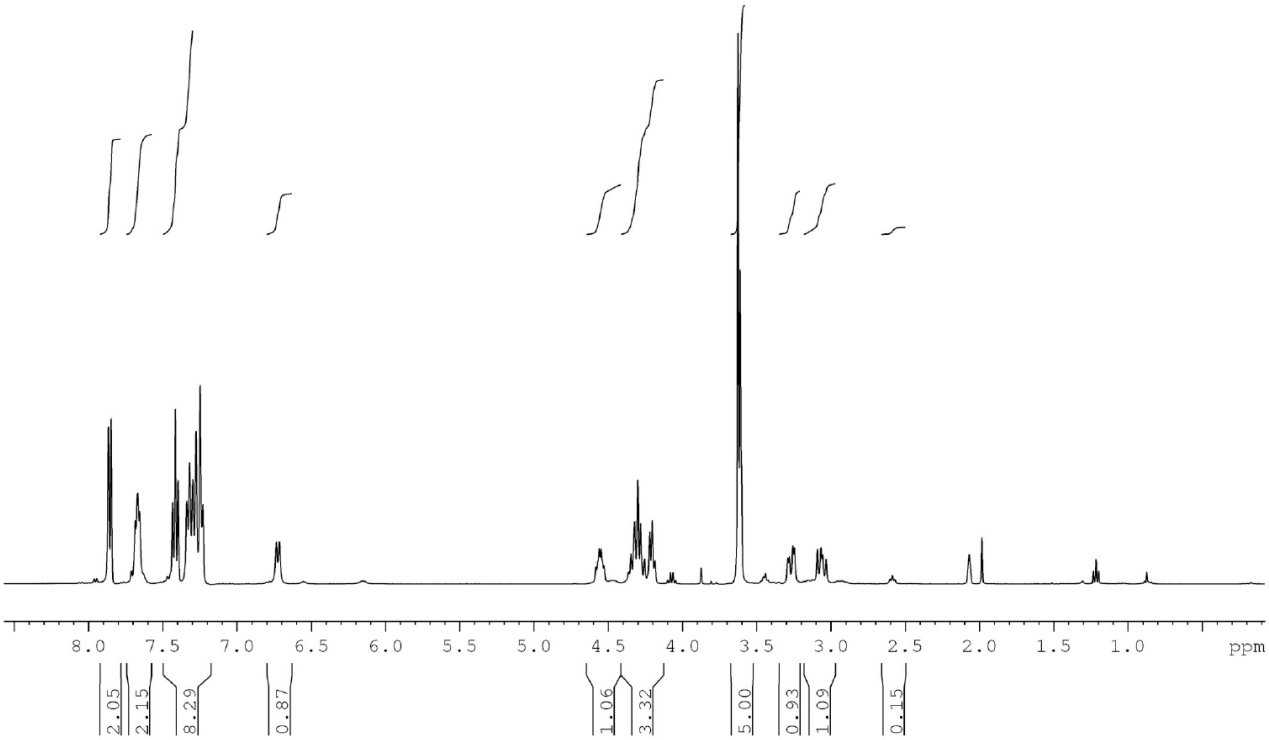
2. Porter, C. J.; Wilce, M. C.; Mackay, J. P.; Leedman, P.; Wilce, J. A. Grb7-SH2 domain dimerisation is affected by a single point mutation. *Eur. Biophys. J.* **2005,** *34*, 454-60.

3. Bradford, M. M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical biochemistry* **1976,** *72*, 248-54.

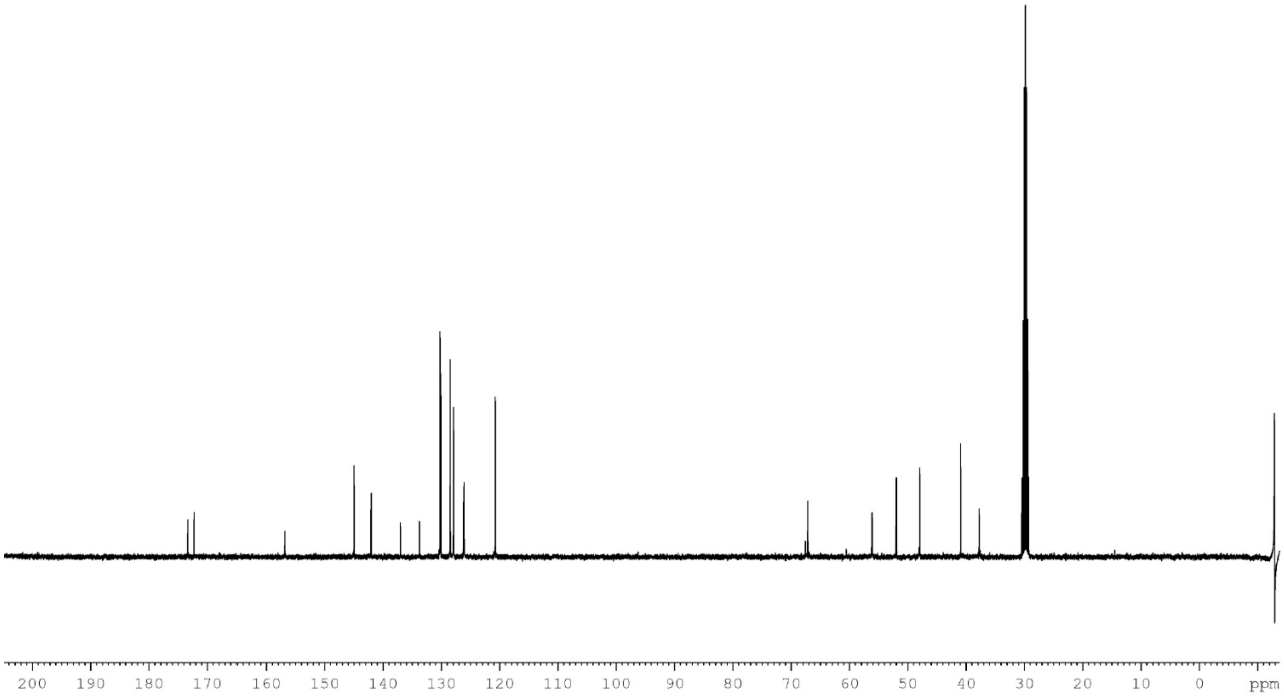
4. Gunzburg, M. J.; Ambaye, N. D.; Hertzog, J. T.; Borgo, M. P.; Pero, S. C.; Krag, D. N.; Wilce, M. C. J.; Aguilar, M.-I.; Perlmutter, P.; Wilce, J. A. Use of SPR to Study the Interaction of G7-18NATE Peptide with the Grb7-SH2 Domain. *Int. J. Pept. Res. Ther.* **2010,** *16*, 177-184.

II ^{1}H and ^{13}C NMR spectra of Fmoc-cmF(OMe)-OH **4**

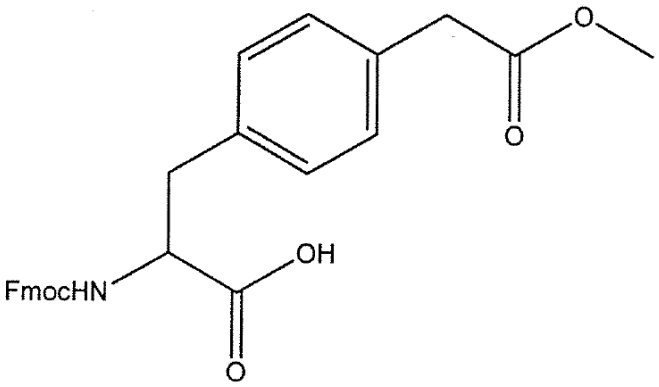
^{1}H NMR, 400 MHz, d^{6}-acetone



^{13}C NMR, 100 MHz, d^{6}-acetone



III ms for Fmoc-cmF(OMe)-OH **4**

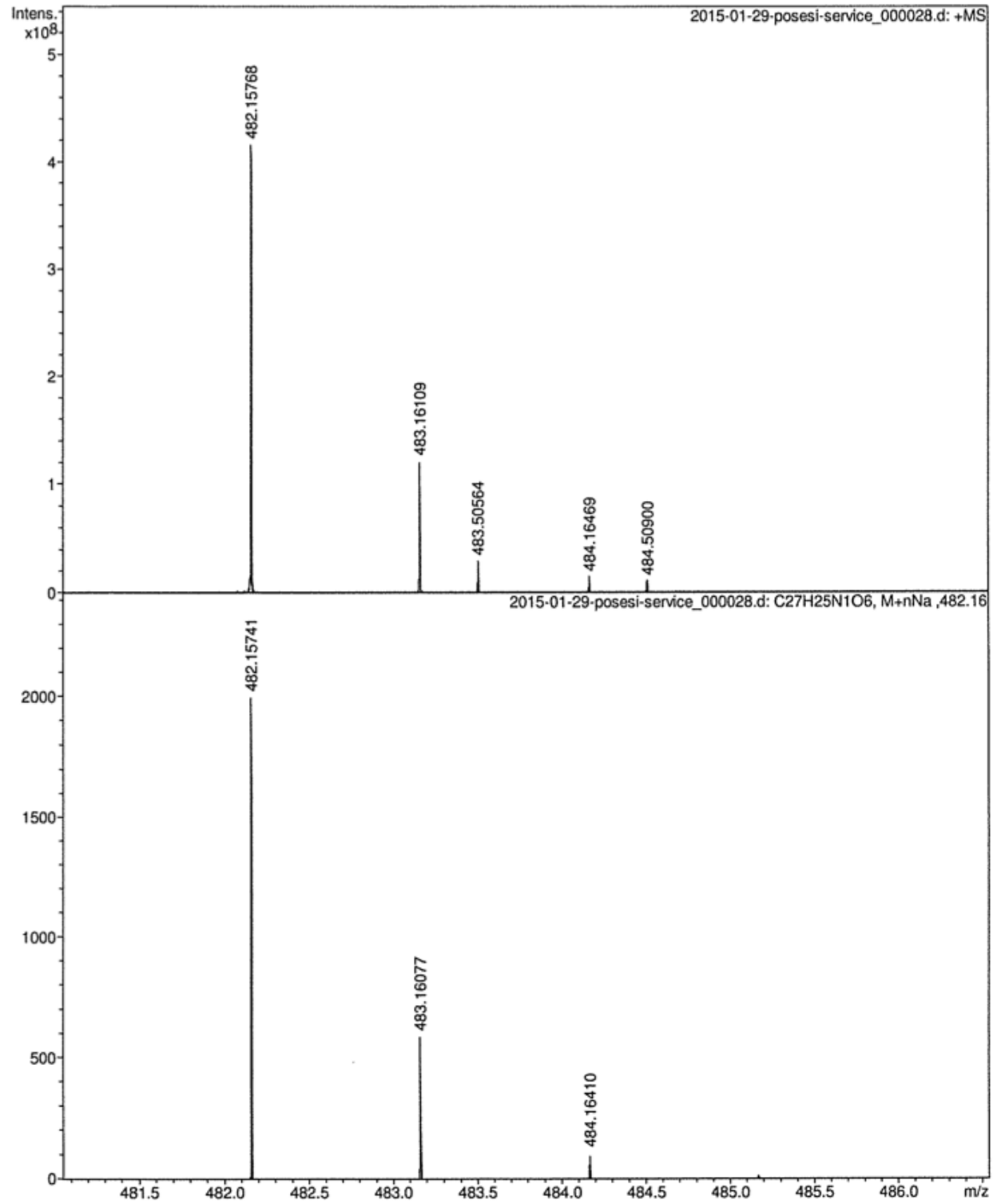


Chemical Formula: $C_{27}H_{25}NO_6$
Exact Mass: 459.1682
Molecular Weight: 459.4905
m/z: 459.1682 (100.0%), 460.1715 (29.2%), 461.1749 (4.1%), 461.1724 (1.2%)
Elemental Analysis: C, 70.58; H, 5.48; N, 3.05; O, 20.89

Generic Display Report

Analysis Info

		Acquisition Date	29/01/2015 4:24:32 PM
Analysis Name	D:\Data\Nick-2015-files\ESI_Positive\01-January\2015-01-29\2015-01-29-posesi-service_000028.d		
Method	1MW Positive ESI	Operator	
Sample Name	Fmoc CarboxyMePhe_OMe	Instrument	apex-Ultra
Comment	MeOH 1M TOF delay 0.0005s, Q1 200 m/z		

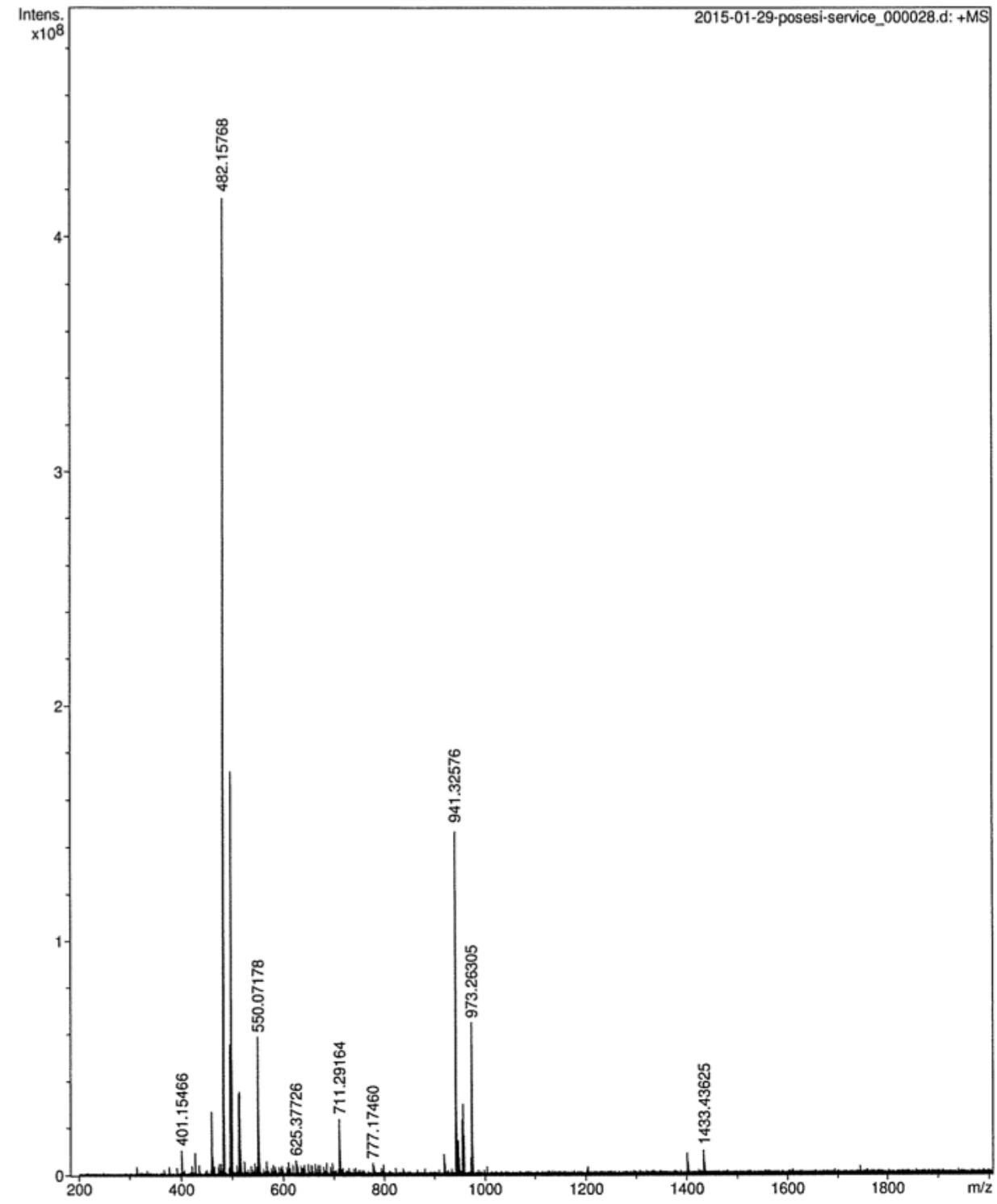


Bruker Compass DataAnalysis 4.0 printed: 29/01/2015 4:33:33 PM Page 1 of 1

Generic Display Report

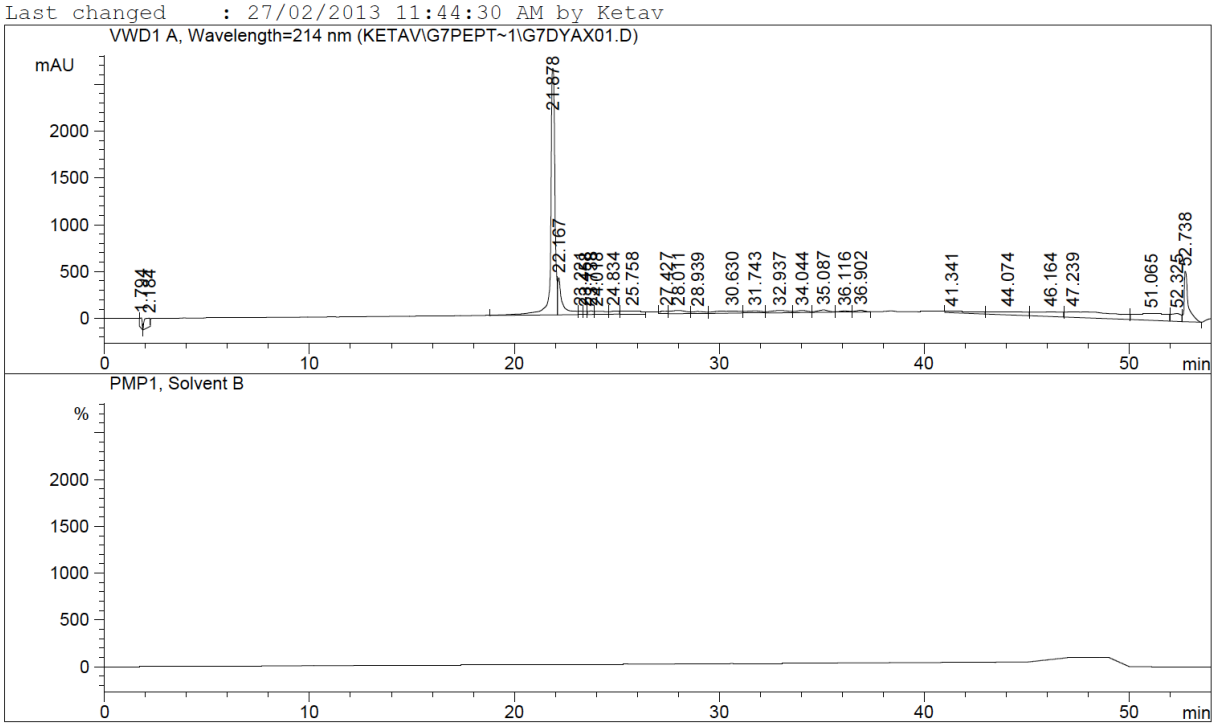
Analysis Info

Acquisition Date	29/01/2015 4:24:32 PM		
Analysis Name	D:\Data\Nick-2015-files\ESI_Positive\01-January\2015-01-29\2015-01-29-posesi-service_000028.d		
Method	1MW Positive ESI	Operator	
Sample Name	Fmoc CarboxyMePhe_OMe	Instrument	apex-Ultra
Comment	MeOH 1M TOF delay 0.0005s, Q1 200 m/z		



IV. LC chromatogram for peptide **2**

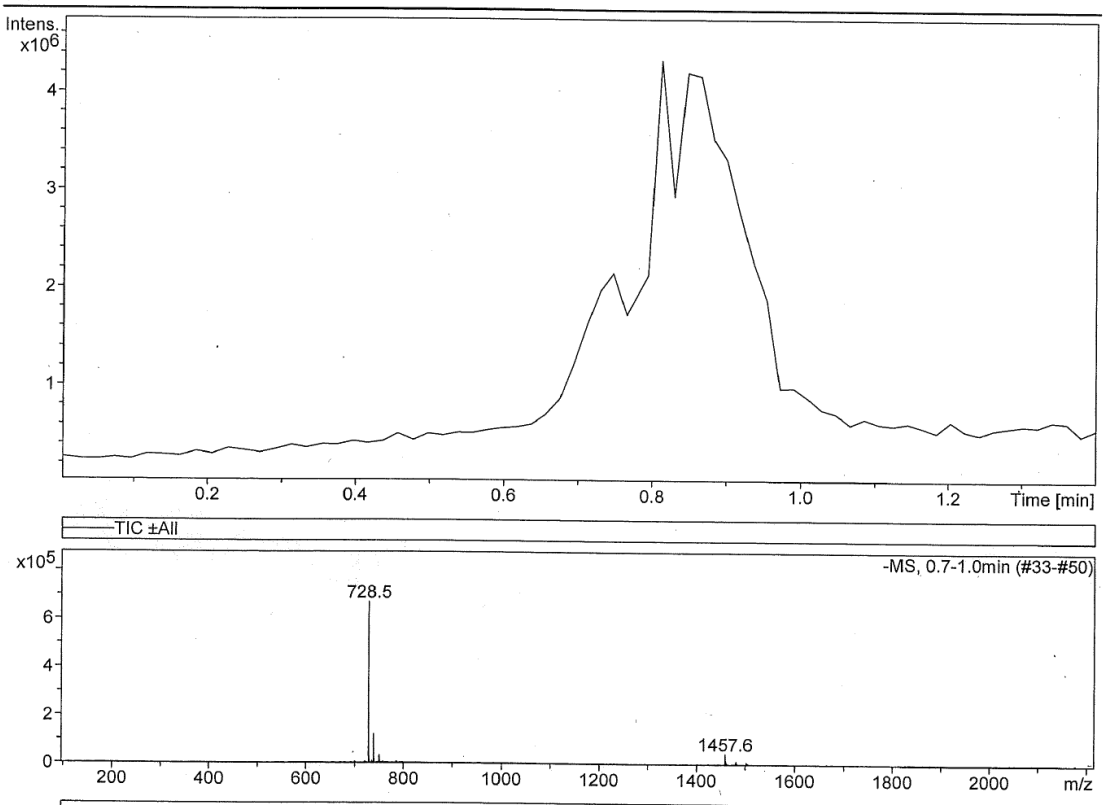
```
Data File C:\HPCHEM\1\DATA\KETAV\G7PEPT~1\G7DYAX01.D              Sample Name: G7-Y+carboxyFTE

======================================================================
Injection Date  : 27/02/2013 12:57:44 PM         Seq. Line :   1
Sample Name     : G7-Y+carboxyFTE                 Location : Vial 1
Acq. Operator   : Ketav                              Inj :   1
Acq. Instrument : Instrument 1                  Inj Volume : 25 µl
Different Inj Volume from Sequence !     Actual Inj Volume : 28 µl
Method          : C:\HPCHEM\1\METHODS\SHARON.M
Last changed    : 27/02/2013 11:44:30 AM by Ketav
```



V. ms for peptide **2**

Analysis Name: G7DYAX01.D **Instrument:** LC-MSD-Trap-VL
Method: Copy of SUISMSN.M **Acq. Date:** 02/27/13 14:26:32
Sample Name: G7-Y+XTE

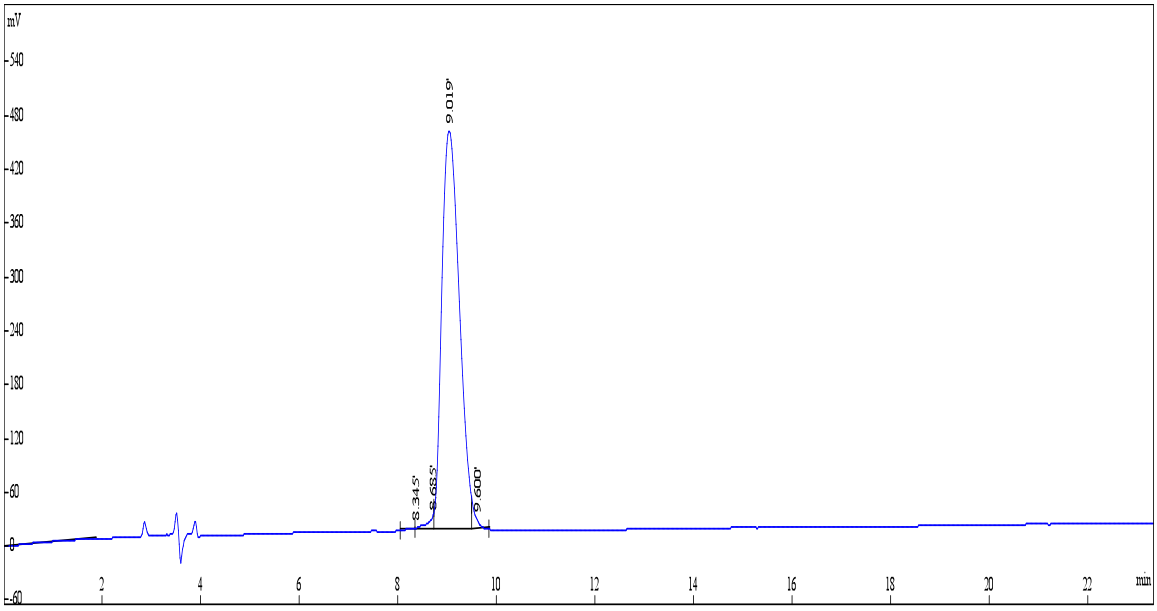
Analysis Info:



VI. LC chromatogram for peptide **3**

HPLC REPORT

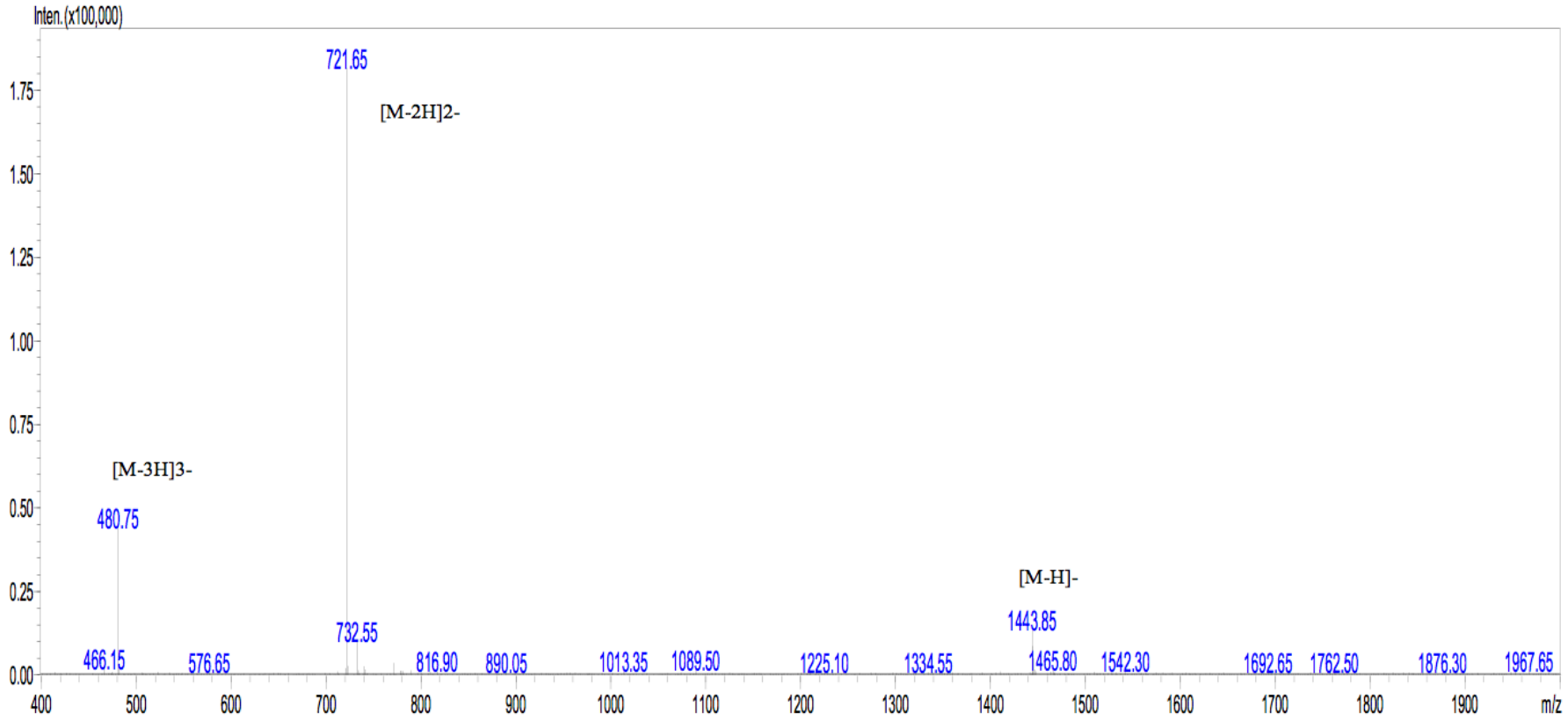
Product Name	:PO 47179501			
Seq	:(cyclo-(CH2CO- WFEG(carboxy-phe)DNTFPC(11) -amide)			
Lot No	:P131127-NOD2008			
Column	:Gemini-NX 5μ C18 110A, 4.6*250mm			
Solvent A	:0.1%Trifluoroacetic in 100% Acetonitrile			
Solvent B	:0.1%Trifluoroacetic in 100% Water			
Gradient	:	A	B	
	0.01min	30%	70%	
	25min	55%	45%	
	25.01min	100%	0%	
	30min	Stop		
Flow rate	:1.0ml/min			
Wavelength	:220nm			
Volume	:20ul			



Rank	Time	Conc.	Area
1	8.345	0.2941	33355
2	8.685	1.6644	188800
3	9.019	96.7824	10978283
4	9.600	1.2592	142840
Total		100	11343278

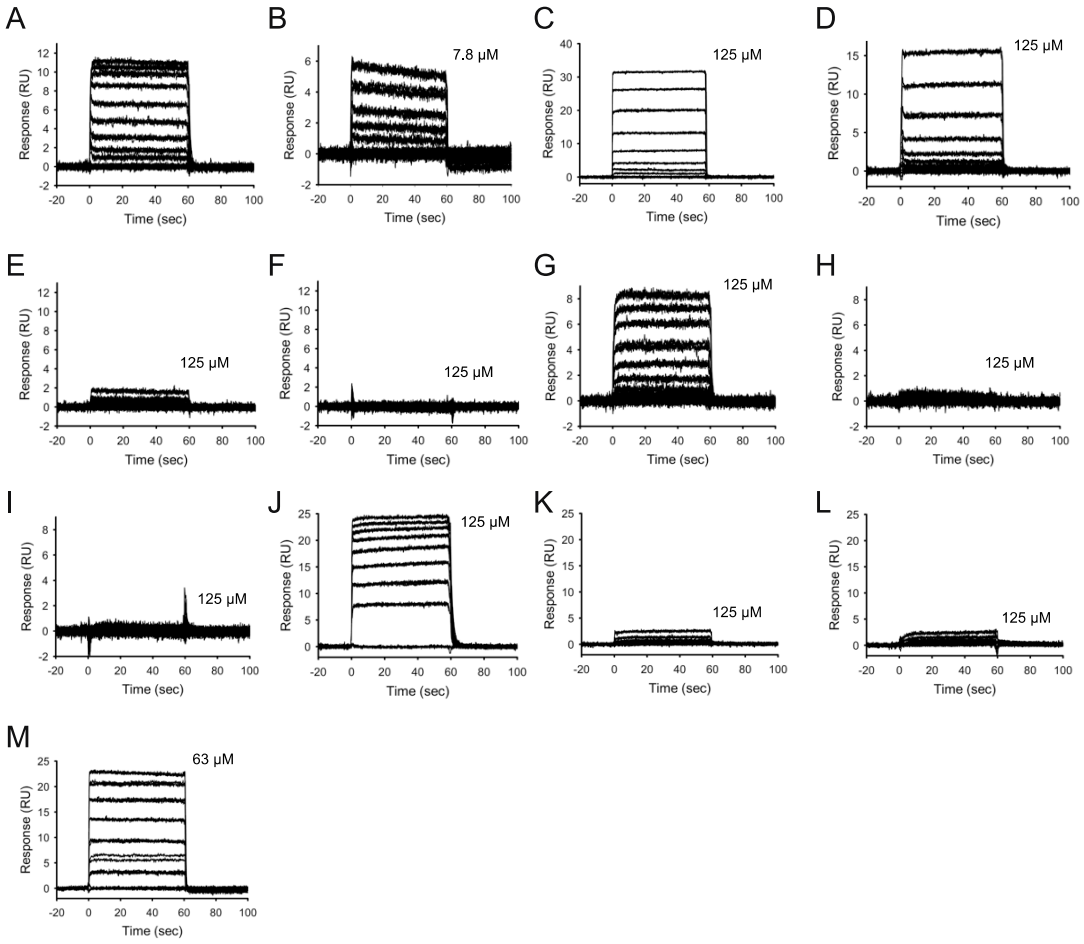
Mass Spectrum

Sequence :



Date and Time:.2013-12-4 12:57:51		Probe:	ESI	Probe bias:	+4.5kv
User	:NOD	Nebulizer Gas Flow:	1.5L/min	Detector:	1.5kv
Sample	:PO 47179501	CDL:	-20.0v	T. Flow:	0.2ml/min
Seq	: (cyclo-(CH2CO- WFEG(carboxy-phe)DNTFPC(11) -amide)	CDL Temp:	250℃	B. conc:	50%H2O/50%ACN
MW	:1445.54	Block Temp:	200℃		
Lot No.	: P131127-NOD2008				

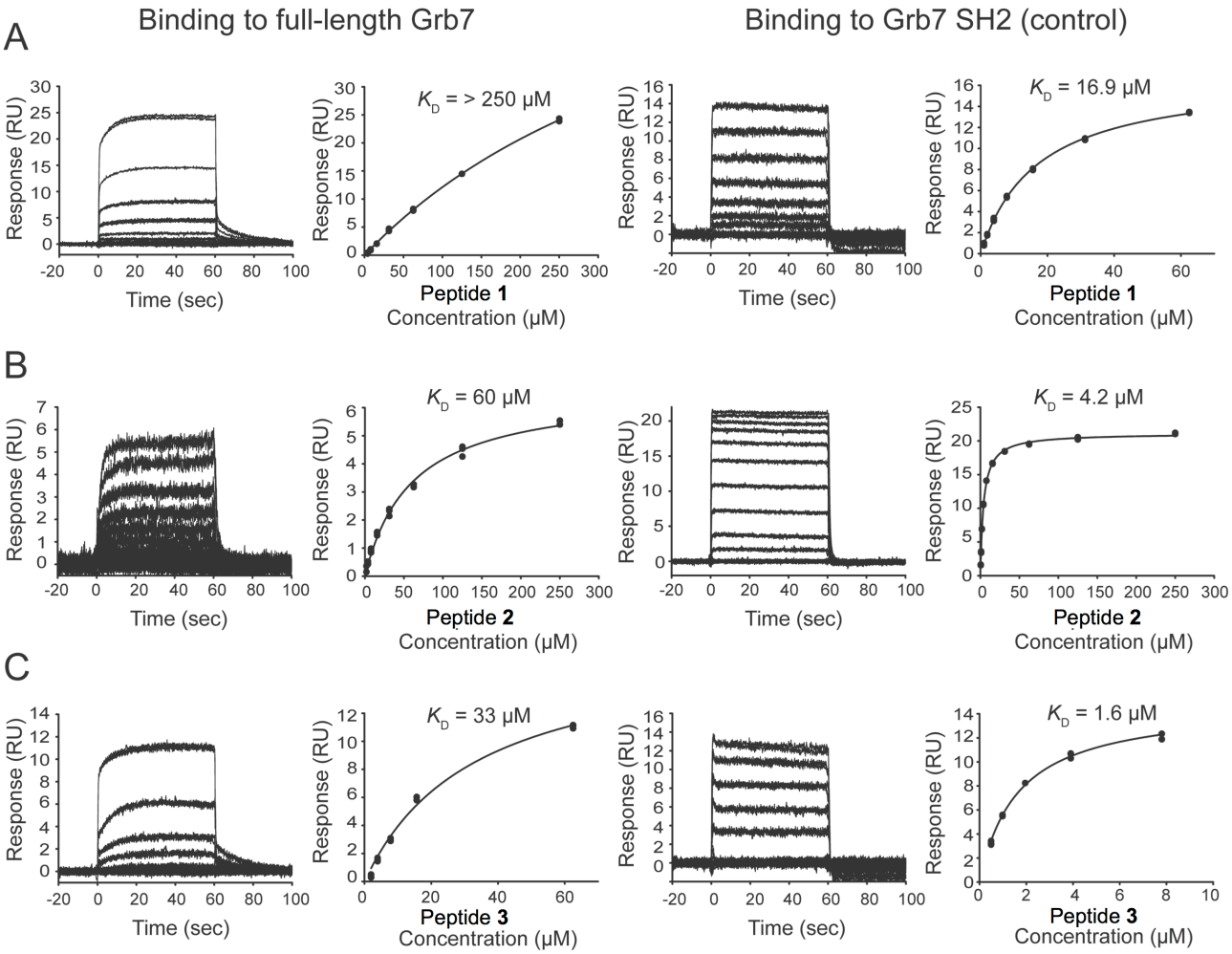
VIII. SPR sensorgrams for **1**, **2** and **3** binding to the SH2 domains of Grb2, Grb7 and Grb10.



SPR sensorgrams acquired for characterising protein:peptide interactions:

(A) Grb7-SH2, 1 mM NaPO4, **2**; (B) Grb7-SH2, 0 mM NaPO4, **2**; (C) Grb7-SH2, 50 mM NaPO4, **2**; (D) Grb7-SH2, high NaPO4, **2**; (E) Grb2-SH2, 1 mM NaPO4, **2**; (F) Grb10-SH2, 1 mM NaPO4, **2**; (G) Grb7-SH2, 1 mM NaPO4, peptide **1**; (H) Grb2-SH2, 1 mM NaPO4, peptide **1**; (I) Grb10-SH2, 1 mM NaPO4, peptide **1**; (J) Grb7-SH2, 1 mM NaPO4, **3**; (K) Grb2-SH2, 1 mM NaPO4, **3**; (L) Grb10-SH2, 1 mM NaPO4, **3**; (M) Grb7-SH2, 50 mM NaPO4, **3**.

IX. SPR sensorgrams and equilibrium binding curves for **1**, **2** and **3** binding to full-length Grb7 and Grb7-SH2



SPR sensorgrams acquired and equilibrium binding curves generated for characterising protein: peptide interactions: (A) Peptide **1** binding to full-length (left) and the Grb7-SH2 domain (right); (B) peptide **2** binding to full-length (left) and the Grb7-SH2 domain (right); (C) peptide **3** binding to full-length (left) and the Grb7-SH2 domain (right); The data show that all three peptides bind to full-length Grb7 with affinity to Grb7 in the order of peptide **3** > peptide **2** > peptide **1**. Simultaneous binding experiments to Grb7-SH2 were run as positive controls.