

1. Sample description

1.1. SIR2RP1 protein

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      10          20          30          40          50          60
MARGSHHHHH HLEMTASPRA PHQEHLVLP TLEGLAHYIR EKNVRRILVL VGAGASVAAG

      70          80          90          100         110         120
IPDFRSPDTG IYANLGKYNL EDPTDAFSLT LLREKPEIFY SIARELNLWP GHFQPTAVHH

      130         140         150         160         170         180
FIRLLQDEGR LLRCCTQNID GLEKAAGVSP ELLVEAHGSF AAAACIECHT PFSIEQNYLE

      190         200         210         220         230         240
AMSGTVSRCS TCGGIVKPNV VFFGENLPDA FFDALHHDAP IAELV I IIGT SMQVHPFALL

      250         260         270         280         290         300
PCVVPKSVPR VVMNRERVGG LLFRFPDDPL NTVHEDAVAK EGRSSSSQSR SPSASPRREE

      310         320         330         340         350         360
GGTEDSPSSP NEEVEEASTS SSSDGYGYG DYHAHPDVCR DVLFRGDCQE NVVTLAEYLG

      370         380
LSEALAKRMR LSDAAPATAQ RAPNET
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Molecular weight = 42112.13

Residues = 386

Isoelectric Point = 5.7

Extinction Coefficient = 17210

2. Experimental section

2.1. LC-UV/MS

HPLC system: Alliance 2695 (Waters)

UV detector: PDA 2996 (Waters)

Column	XBRIDGE BEH300 C4 3.5 μ m (2.1 \times 50mm)
Column temperature	50°C
Flow rate	0.2 ml/min
Gradient	5% to 90% B in 30 min
Mobile phase A	0.05% TFA/0.1% FA/Water
Mobile phase B	0.045% TFA/0.1% FA/70% ACN/30% iPrOH
Diode Array Detection	200-400 nm

HPLC system was coupled to ESI-TOF mass spectrometer (LCT, Waters) equipped with the Triversa Nanomate (Advion) microfluidic nano-ESI device. Mass spectrometer was operated in the positive ion mode in the mass range m/z 500-5000.

2.2. Peptide assignment

BioLynx was used to assign the protein cleavage products detected by LC-UV/MS. Search criteria included trypsin digestion specificity, mass tolerance was set at 0.6 Da for peptides smaller than 10kDa, and at 1 Da for large protein products (> 20kDa).

3. Results

LC-UV analysis of SIR2RP1 submitted to limited trypsin digestion provided LC profile depicted in figure 1. Two very low intensity peaks (①) eluted first from the BEH column (8.99 and 9.53 min). Two major peaks, ② and ③, eluted at 14.84 min and 20.29 min, respectively.

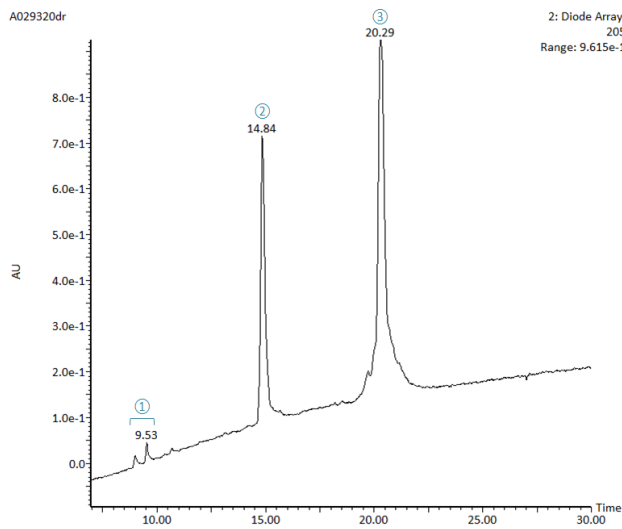
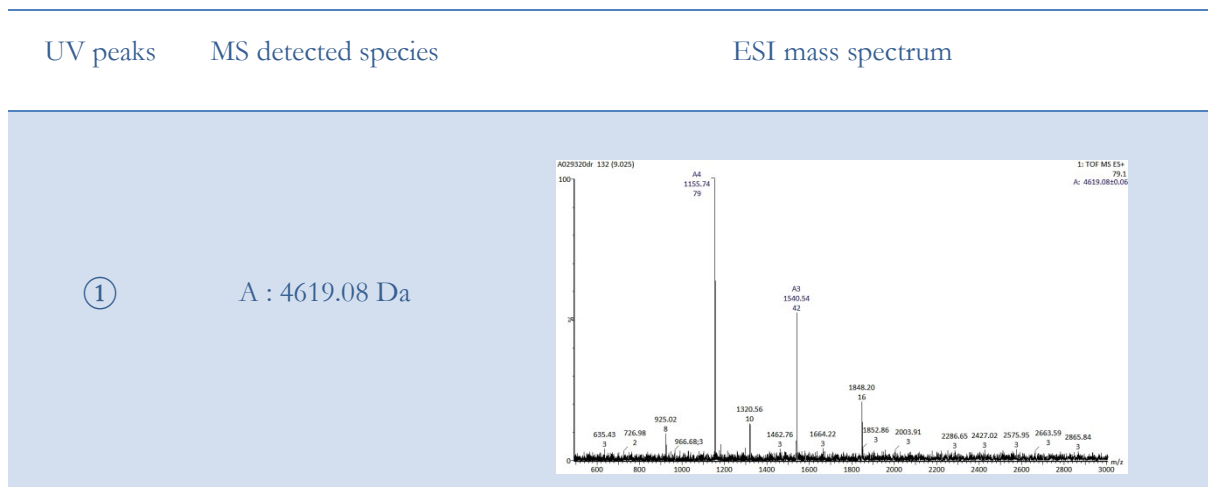
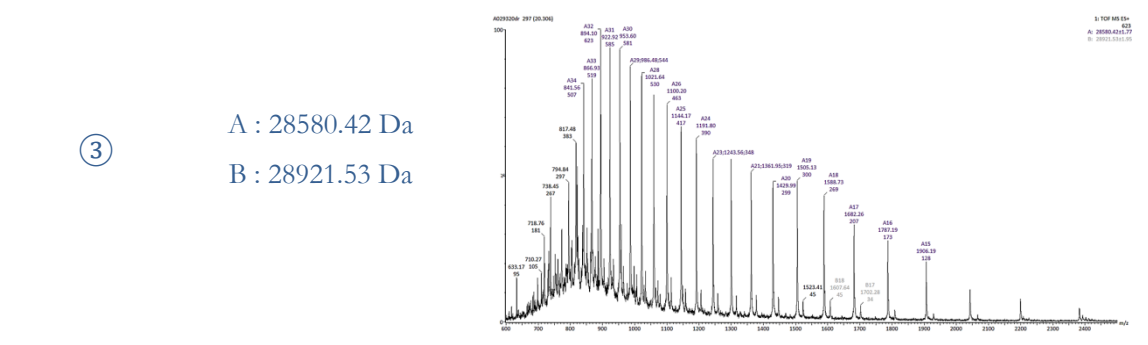
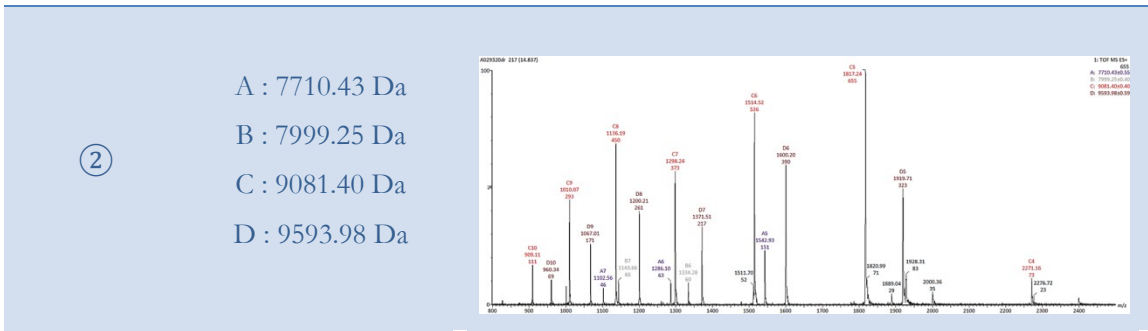


Figure 1: LC elution profile of SIR2RP1 sample following limited trypsin proteolysis.

MS analysis revealed a number of species eluting in the UV-detected peaks:





Peptide assignment was performed as described in the experimental section unless specified otherwise in the table below.

Peak ①

Species "A" : 4619.08 Da

Hit #	Res#	Mass	Sequence	C-Term	Total Mass	Dev	S-S
1	298-340	4619.61	(R) REGGTRDSDSPSPNEEV EASATSSSDGTYGQYDHYA HPDVCRLFRGDCQENVVT LAEYLGLSEALAKR (D)	-OH	4619.61	0.53	-

Peak ②

Species "A" : 7710.43 Da

First peptide mass search with done with a mass tolerance of 0.6 Da and trypsin specificity provided no results. Mass tolerance was then increased to 2 Da while keeping trypsin digestion specificity. With these search criteria, a single peptide hit was found but would assume one disulfide bridge to explain the unusually large mass error (1.71 Da).

Hit #	Res#	Mass	Sequence	C-Term	Total Mass	Dev	S-S
3	298-368	7712.14	(R) REGGTRDSDSPSPNEEV EASATSSSDGTYGQYDHYA HPDVCRLFRGDCQENVVT LAEYLGLSEALAKR (H)	-OH	7712.14	1.71	-

Species "B" : 7999.25 Da

Hit #	Res#	Mass	Sequence	C-Term	Total Mass	Dev	S-S
1	298-370	7999.52	(R) REGGTRDSDSPSPNEEV EASATSSSDGTYGQYDHYA HPDVCRLFRGDCQENVVT LAEYLGLSEALAKR (L)	-OH	7999.52	0.27	-

Peak ②

Species "C" : 9081.40 Da

Mass tolerance: 0.60
Number of hits = 2
Hit# Res# Mass Sequence
* 2 298-381 9081.71 (R)REKGGTEDEPSPFNNEV
EEAFTSSSSDGYQGDYHA
HFDVCRDVLFRGDCQENVVF
LAETLGLSEALAGNRLSDA
APATAQR(A)

C-Term -OH
Total Mass 9081.71
Dev 0.31
S-S -

1 MARGS HHHH HLEMT ASPRA FQEH VLGEF TLEGL AHYIR
41 EKQVR RILVL VGAGA SVAAG IDQFR SFGTG IYANL GKYNL
81 EDPTD AFSLT LREK PEIFY STARE LNLMF GHPQF TAVNH
121 FIRLL QDEGR LREK TQNIID GLEKA AGVSP ELIWE AHGSP
161 AAAAC IECIT PFSIE QNYLE AMSGT VSRCS TCGGI VKPNV
201 VFFGE NLFDA FFDAL HHDAP IAEVL IITGT SMQVH PFALL
241 PCVVF KSVPR VVNHK ERVGG LLFRF DDQPL NVVHE DAVAK
281 EGRSS SGRSR SPSAS FRRSE GDTED SPSSE NEEVE EASTS
321 SSSDG YQGYG DYHAM DVQCR DVLFK GDCQE NVVTL AYLIG
361 LREAL AKRMR LQDAA PATAQ RAINE T

Species "D" : 9593.98 Da

Mass tolerance: 0.60
Number of hits = 1
Hit# Res# Mass Sequence
* 1 298-386 9594.23 (R)REKGGTEDEPSPFNNEV
EEAFTSSSSDGYQGDYHA
HFDVCRDVLFRGDCQENVVF
LAETLGLSEALAGNRLSDA
APATAQR(A)

C-Term -OH
Total Mass 9594.23
Dev 0.25
S-S -

1 MARGS HHHH HLEMT ASPRA FQEH VLGEF TLEGL AHYIR
41 EKQVR RILVL VGAGA SVAAG IDQFR SFGTG IYANL GKYNL
81 EDPTD AFSLT LREK PEIFY STARE LNLMF GHPQF TAVNH
121 FIRLL QDEGR LREK TQNIID GLEKA AGVSP ELIWE AHGSP
161 AAAAC IECIT PFSIE QNYLE AMSGT VSRCS TCGGI VKPNV
201 VFFGE NLFDA FFDAL HHDAP IAEVL IITGT SMQVH PFALL
241 PCVVF KSVPR VVNHK ERVGG LLFRF DDQPL NVVHE DAVAK
281 EGRSS SGRSR SPSAS FRRSE GDTED SPSSE NEEVE EASTS
321 SSSDG YQGYG DYHAM DVQCR DVLFK GDCQE NVVTL AYLIG
361 LREAL AKRMR LQDAA PATAQ RAINE T

Peak ③

Species "A" : 28580.43 Da

Mass tolerance: 1.00
Number of hits = 1
Hit# Res# Mass Sequence
* 1 20-280 28579.86 (R)APQHEVLRGPTLEGIA
HYIREQNRRLIVLVGAS
VVAAGIDPFRSFGTGYANLG
KYNLEDPTDFAFSLTLREKQ
EIFYSIARELNLMGHPQPT
AVHHPTRLLGQGRLLKCCP
QNIIDGKAGVSEFELIWEA
HSPAAACIECITPFSIEQ
NYLEAMGTVSRCSFCGGIV
KRVVFFGKMLGDAFFLAH
RDAPIAELVIIIGTSMQVH
FALLPCVVKSVPRVVMNRE
RVGGLLEFFFGDPLNVVHE
AVAK(E)

C-Term -OH
Total Mass 28579.86
Dev -0.57
S-S -

1 MARGS HHHH HLEMT ASPRA FQEH VLGEF TLEGL AHYIR
41 EKQVR RILVL VGAGA SVAAG IDQFR SFGTG IYANL GKYNL
81 EDPTD AFSLT LREK PEIFY STARE LNLMF GHPQF TAVNH
121 FIRLL QDEGR LREK TQNIID GLEKA AGVSP ELIWE AHGSP
161 AAAAC IECIT PFSIE QNYLE AMSGT VSRCS TCGGI VKPNV
201 VFFGE NLFDA FFDAL HHDAP IAEVL IITGT SMQVH PFALL
241 PCVVF KSVPR VVNHK ERVGG LLFRF DDQPL NVVHE DAVAK
281 EGRSS SGRSR SPSAS FRRSE GDTED SPSSE NEEVE EASTS
321 SSSDG YQGYG DYHAM DVQCR DVLFK GDCQE NVVTL AYLIG
361 LREAL AKRMR LQDAA PATAQ RAINE T

Species "B" : 28921.53 Da

Mass tolerance: 1.00
Number of hits = 1
Hit# Res# Mass Sequence
* 1 20-283 28922.21 (R)APQHEVLRGPTLEGIA
HYIREQNRRLIVLVGAS
VVAAGIDPFRSFGTGYANLG
KYNLEDPTDFAFSLTLREKQ
EIFYSIARELNLMGHPQPT
AVHHPTRLLGQGRLLKCCP
QNIIDGKAGVSEFELIWEA
HSPAAACIECITPFSIEQ
NYLEAMGTVSRCSFCGGIV
KRVVFFGKMLGDAFFLAH
RDAPIAELVIIIGTSMQVH
FALLPCVVKSVPRVVMNRE
RVGGLLEFFFGDPLNVVHE
AVAKGR(S)

C-Term -OH
Total Mass 28922.21
Dev 0.68
S-S -

1 MARGS HHHH HLEMT ASPRA FQEH VLGEF TLEGL AHYIR
41 EKQVR RILVL VGAGA SVAAG IDQFR SFGTG IYANL GKYNL
81 EDPTD AFSLT LREK PEIFY STARE LNLMF GHPQF TAVNH
121 FIRLL QDEGR LREK TQNIID GLEKA AGVSP ELIWE AHGSP
161 AAAAC IECIT PFSIE QNYLE AMSGT VSRCS TCGGI VKPNV
201 VFFGE NLFDA FFDAL HHDAP IAEVL IITGT SMQVH PFALL
241 PCVVF KSVPR VVNHK ERVGG LLFRF DDQPL NVVHE DAVAK
281 EGRSS SGRSR SPSAS FRRSE GDTED SPSSE NEEVE EASTS
321 SSSDG YQGYG DYHAM DVQCR DVLFK GDCQE NVVTL AYLIG
361 LREAL AKRMR LQDAA PATAQ RAINE T