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Supporting Information

Screen and Optimization of Phage Display Cyclic Peptides Against WDR5 WBM Site

Lingyu Song,a,b Jiawen Cao,a,b Lin Chen,b Zhiyan Du,b Naixia Zhang,a,c Danyan Cao*b and Bing Xiong*a,b,d

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[a] Department of College of Pharmacy, University of Chinese Academy of Sciences, 19A Yuquan Road, Beijing 100049, China.

Email: bxiong@simm.ac.cn (B. Xiong); caody@simm.ac.cn (D. Cao); s20-songlingyu@simm.ac.cn (L. Song)

[b] Department of Medicinal Chemistry, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, 555 Zuchongzhi Road, Shanghai 201203, China

[c] Department of Analytical Chemistry, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, 555 Zuchongzhi Road, Shanghai 201203, China

[d] State Key Laboratory of Chemical Biology, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, 555 Zuchongzhi Road, Shanghai 201203, China

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Table S1. Phage titer and recovery efficiency of three rounds in the biopanning; recovery efficiency = output titer / input titer

Round	Input Titer (pfu)	Output Titer (pfu)	Recovery Efficiency	Fold Increase
Subtractive panning with Ni-NTA magnetic agarose beads				
1	1.0×10^{11}	1.0×10^{4}	1.0×10^{-7}	1
2	2.0×10^{11}	7.0×10^{4}	3.5×10^{-7}	3.5
3	2.0×10^{11}	1.0×10^{4}	0.5×10^{-7}	0.5
Alternant panning with two kinds of magnetic beads				
1	1.0×10^{11}	2.0×10^{6}	2.0×10^{-3}	1
2	2.0×10^{11}	4.0×10^{5}	2.0×10^{-4}	0.1
3	2.0×10^{11}	2.5×10^{8}	1.25×10^{-1}	62.5

Table S2. The peptide sequences derived from the third round in the subtractive panning; *: the sequence with an affinity value lower than -21.0

No.	Peptide Sequence	Reads (Sanger)	PSBinder (SAROTUP)	Affinity (ADCP)
A1	CAHQRWVTC	4/33	0.93	-
A2	CPWQVHRDC	3/33	0.53	error
A3	***CADTNWLVC**	2/33	0.52	**-22.7**
A4	CSSNTVPAC	2/33	0.04	-19.6
A5	CPWKTHQSC	2/33	0.17	error
A6	CSEARWMRC	2/33	0.12	-20.2
A7	***CSQAWWYQC**	1/33	0.24	**-22.7**
A8	CPWKVHSYC	1/33	0.81	-
A9	***CPNQWKSYC**	1/33	0.27	**-22.0**
A10	CNNLNAILC	1/33	0.08	-20.2
A11	***CFLNVSNAC**	1/33	0.25	**-21.1**
A12	CDMPPTQKC	1/33	0.55	-19.7
A13	CWHAGRWTC	1/33	0.78	-
A14	CLPNDHLRC	1/33	0.20	error
A15	CGSSRWLEC	1/33	0.79	-

Table S3. The peptide sequences derived from the third round in the alternant panning; *: the sequence with an affinity value lower than -21.0

No.	Peptide Sequence	Reads (Sanger)	PSBinder (SAROTUP)	Affinity (ADCP)
B1	***CGSLDWPHC**	5/124	0.69	**-22.8**
B2	CMTPNPTTC	2/124	0.35	-17.2
B3	CRAYDFPSC	2/124	0.97	-
B4	CDARGGLRC	2/124	0.37	-20.2
B5	CRVYEWPKC	1/124	0.98	-
B6	CRALPFNTC	1/124	0.34	-18.6
B7	CRVLPYGSC	1/124	0.94	-
B8	CRVHPVGEC	1/124	0.97	-
B9	CRDKPSNVC	1/124	0.21	-18.7
B10	CGTNPIKKC	1/124	0.27	-17.9
B11	CGSNTTVEC	1/124	0.57	-20.6
B12	CSSNTVPAC	1/124	0.04	-19.6
B13	CVSTATNGC	1/124	0.39	-19.1
B14	***CNSLNWFWC**	1/124	0.30	**-22.2**
B15	CSKKNPSWC	1/124	0.25	-19.1
B16	CYGLSNSRC	1/124	0.40	-19.9
B17	***CGEGEADVC**	1/124	0.50	**-22**
B18	CAIGETSTC	1/124	0.89	-
B19	CLNVSSPTC	1/124	0.10	-18.3
B20	CNNPAQPWC	1/124	0.19	-17.8
B21	CEPRSLANC	1/124	0.10	-19.2
B22	CNWRLSNYC	1/124	0.18	error
B23	CMNEDKFHC	1/124	0.53	error
B24	CTGPWIWHC	1/124	0.96	-
B25	CVNSMTYPC	1/124	0.04	error
B26	CINNLPKSC	1/124	0.12	-19.1
B27	CFPNVYHAC	1/124	0.24	error
B28	CSRSMNMAC	1/124	0.34	-18.6
B29	CHMYHNATC	1/124	0.00	error
B30	CHLTHQASC	1/124	0.00	error

Table S4. Purify and molecular weight of cyclization products with 9 different chemical linkers

Product	Purify	Theoretical M.W.	Calculated M.W.	Observed $[M+H]^+$	Intensity
CYC1	-	1273.50	1274.50	1274.59	1.87E+08
CYC2	-	1273.50	1274.50	1274.52	4.51E+07
CYC3	92.90%	1273.50	1274.50	1274.53	2.43E+06
CYC4	80.98%	1223.49	1224.49	1224.58	5.74E+06
CYC5	-	1225.47	1226.47	1294.37	8.03E+06
CYC6	-	1264.44	1265.44	1462.38	8.17E+06
CYC7	89.00%	1274.50	1275.50	1275.56	4.91E+06
CYC8	-	1269.47	1270.47	1170.51	1.59E+08
CYC9	-	1223.49	1224.49	1170.60	5.70E+07

Table S5. The HPLC gradient elution condition

Time (min)	Mobile Phase A (%)	Mobile Phase B (%)
0.0	20.0	80.0
2.0	95.0	5.0
5.0	60.0	40.0
6.5	5.0	95.0
8.0	5.0	95.0
8.5	95.0	5.0

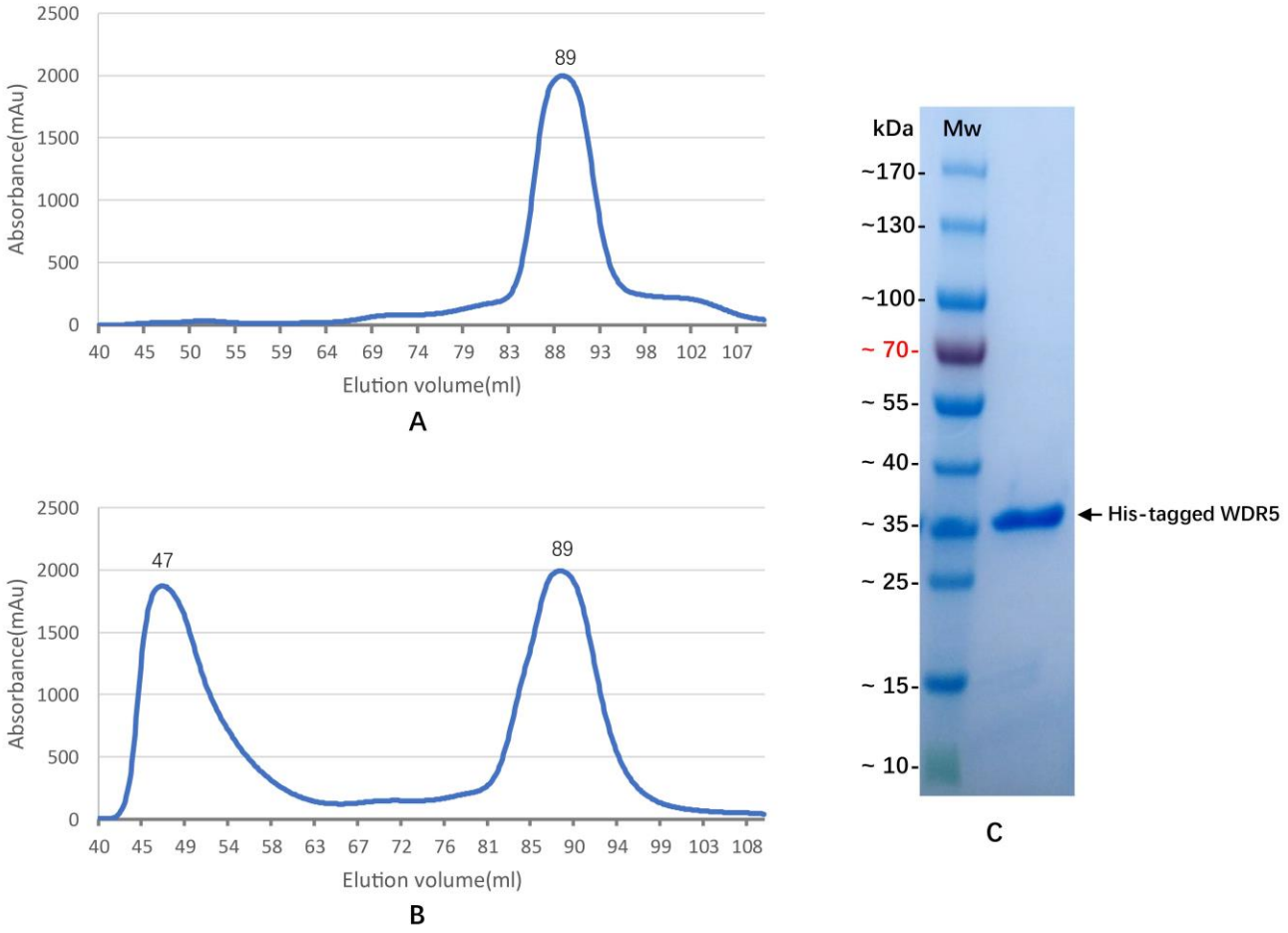


Figure S1. The characterization of purified his-tagged WDR5. (A) Gel-filtration chromatogram of his-tagged WDR5 with a single peak at 89 ml. (B) The elution volume of aggregated WDR5 at 47 ml. (C) SDS-PAGE of purified his-tagged WDR5 protein migrating above the band 35.0 kDa.

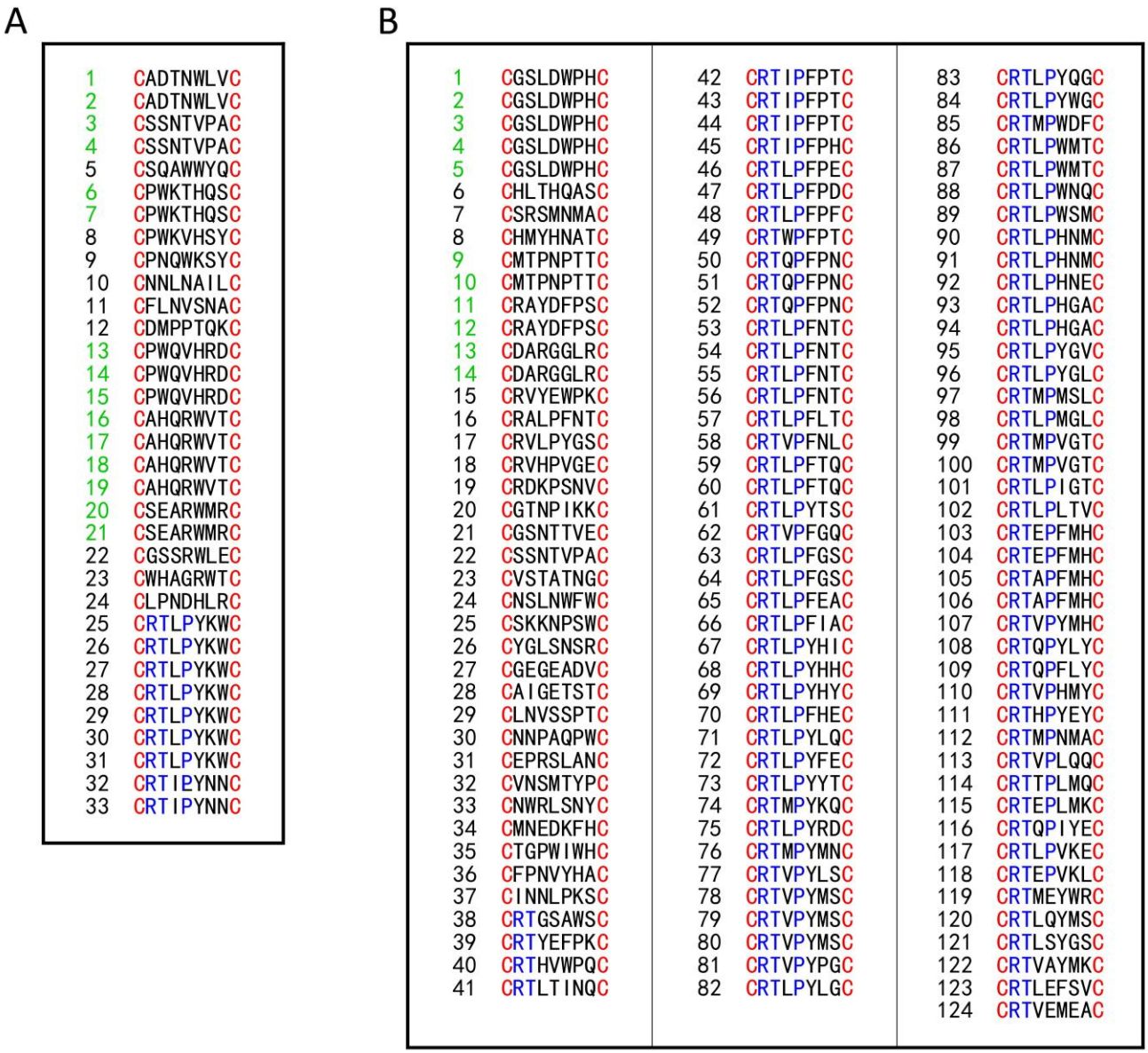


Figure S2. Phage-displayed cyclic peptide sequences picked from the third round of biopanning; numbers of the enriched sequences are marked by green colour. (A) 33 sequences picked from the subtractive panning with Ni-NTA magnetic agarose beads. (B) 124 sequences picked from the alternant panning with two kinds of magnetic beads.

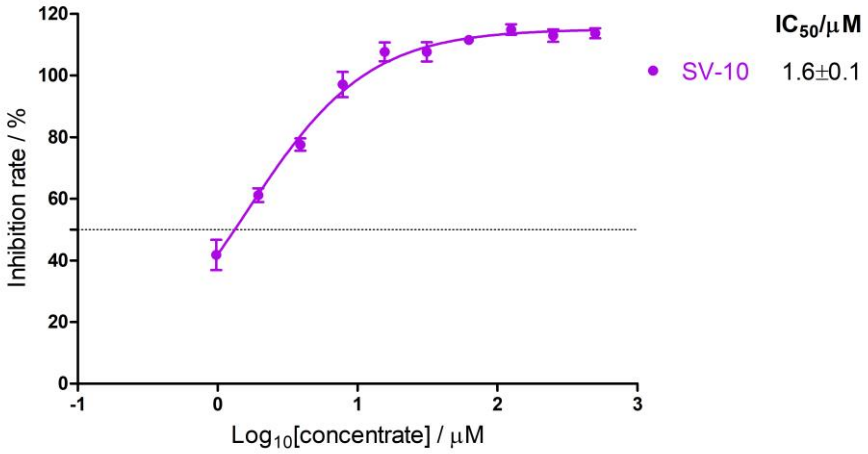
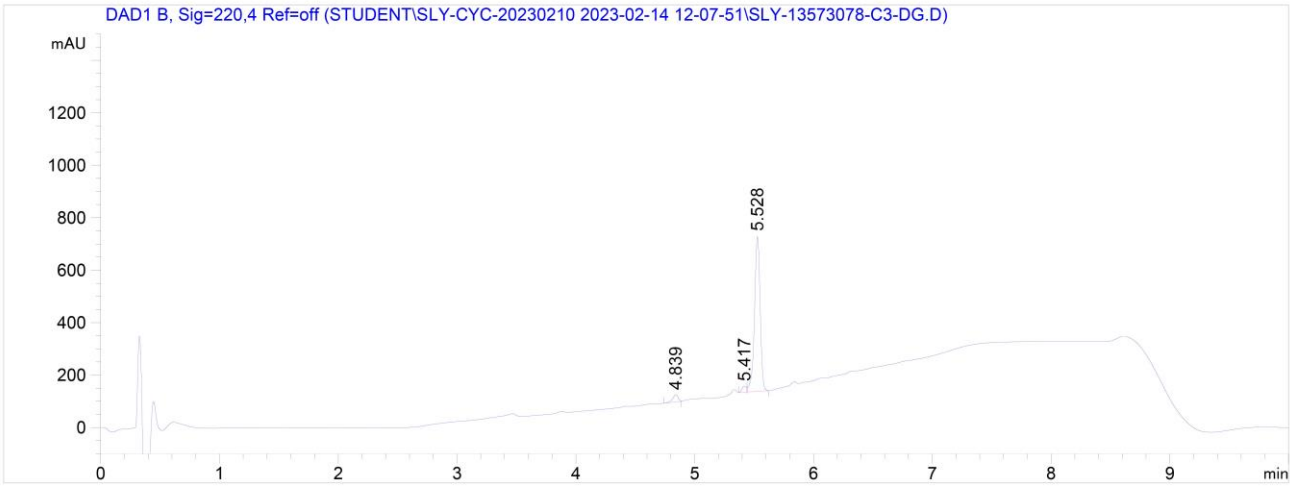


Figure S3. Binding affinity derived from HTRF assay of the positive control peptide SV-10.

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Multiplier            :      1.0000
Dilution              :      1.0000
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 B, Sig=220,4 Ref=off

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   4.839 BV    0.0502   90.97682   27.81858   4.2903
   2   5.417 BV    0.0398   59.60685   23.37117   2.8109
   3   5.528 VB    0.0528 1969.94543  592.72156  92.8988

Totals :                  2120.52910  643.91131
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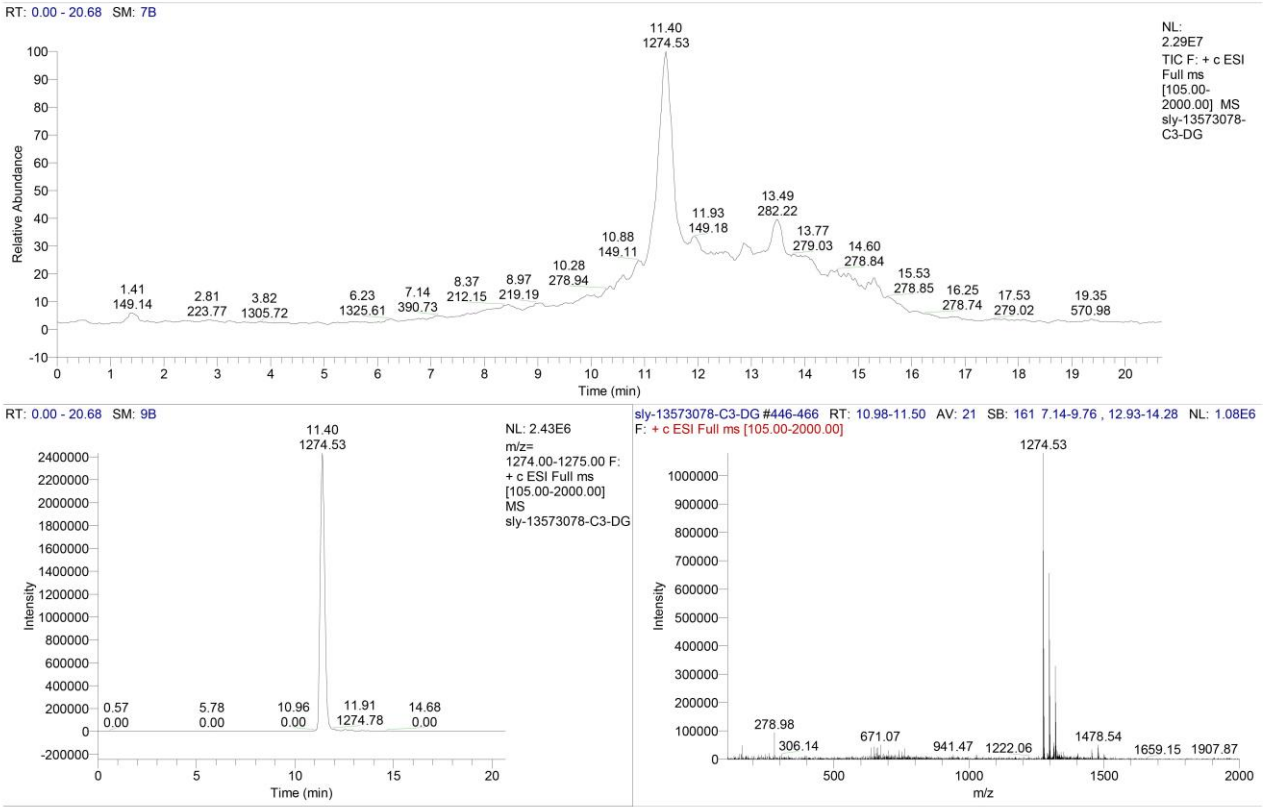
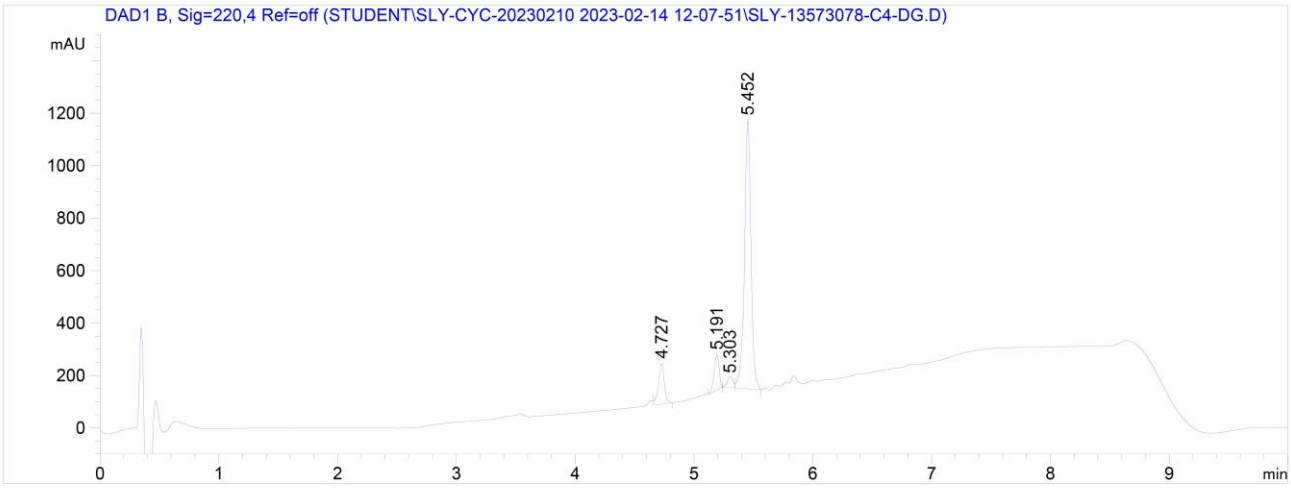


Figure S4. HPLC chromatogram and LC-MS spectrum of the cyclic peptide CYC3.

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Multiplier            :      1.0000
Dilution              :      1.0000
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 B, Sig=220,4 Ref=off

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   4.727 VB    0.0518  529.92902  155.30937   9.6381
   2   5.191 BB    0.0483  393.48483  133.93102   7.1565
   3   5.303 BV    0.0436  122.17971   42.40127   2.2221
   4   5.452 VB    0.0554 3636.68530 1024.22913  80.9833

Totals :                   4682.27886 1355.97079
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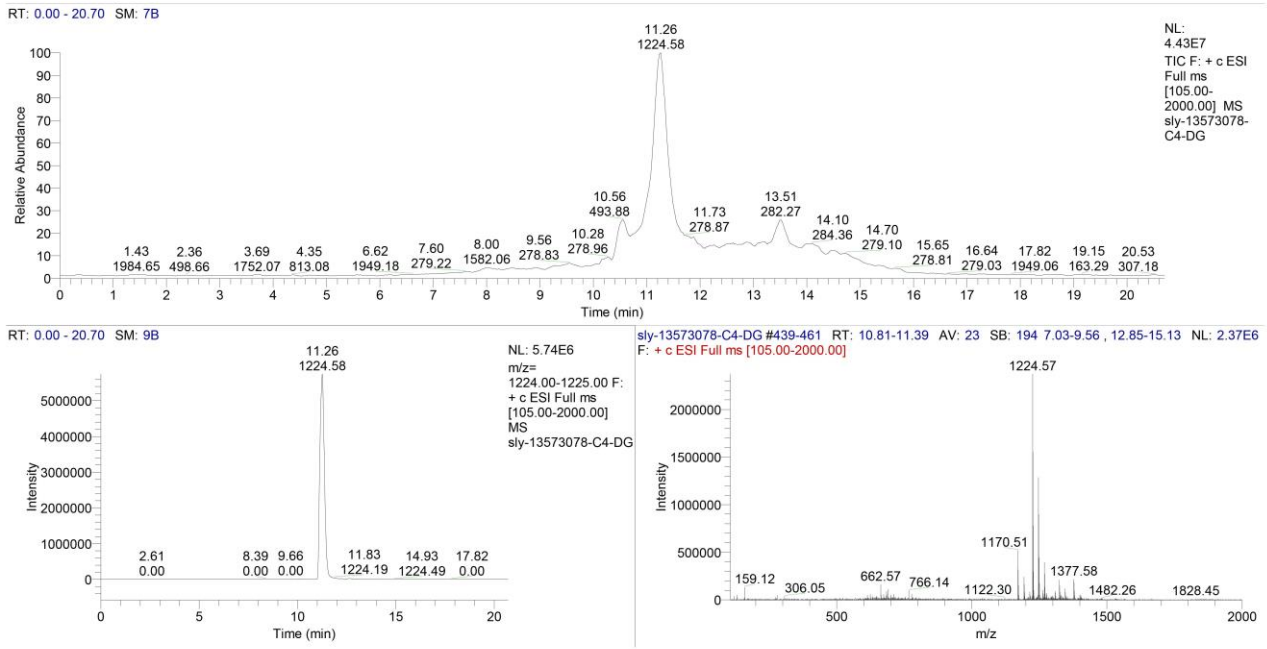
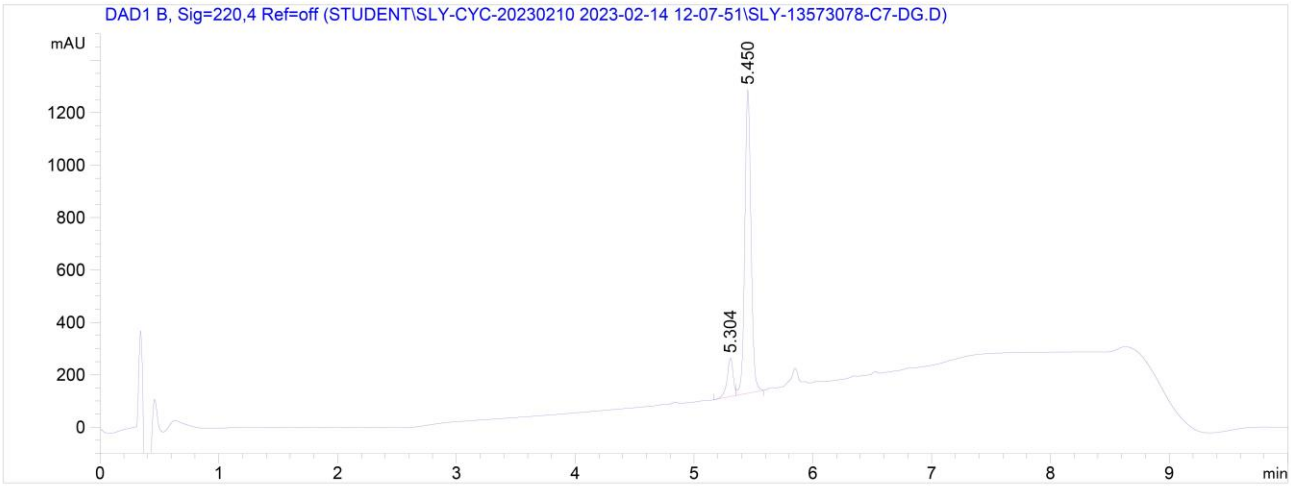


Figure S5. HPLC chromatogram and LC-MS spectrum of the cyclic peptide CYC4.

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Multiplier            :      1.0000
Dilution              :      1.0000
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 B, Sig=220,4 Ref=off

Peak RetTime Type  Width     Area      Height     Area
  #   [min]        [min]   [mAU*s]     [mAU]       %
----|-------|----|-------|----------|----------|--------|
   1   5.304 BV    0.0530  515.54413  146.57593  10.9983
   2   5.450 VB    0.0560 4171.96240 1158.47620  89.0017

Totals :                  4687.50653 1305.05212
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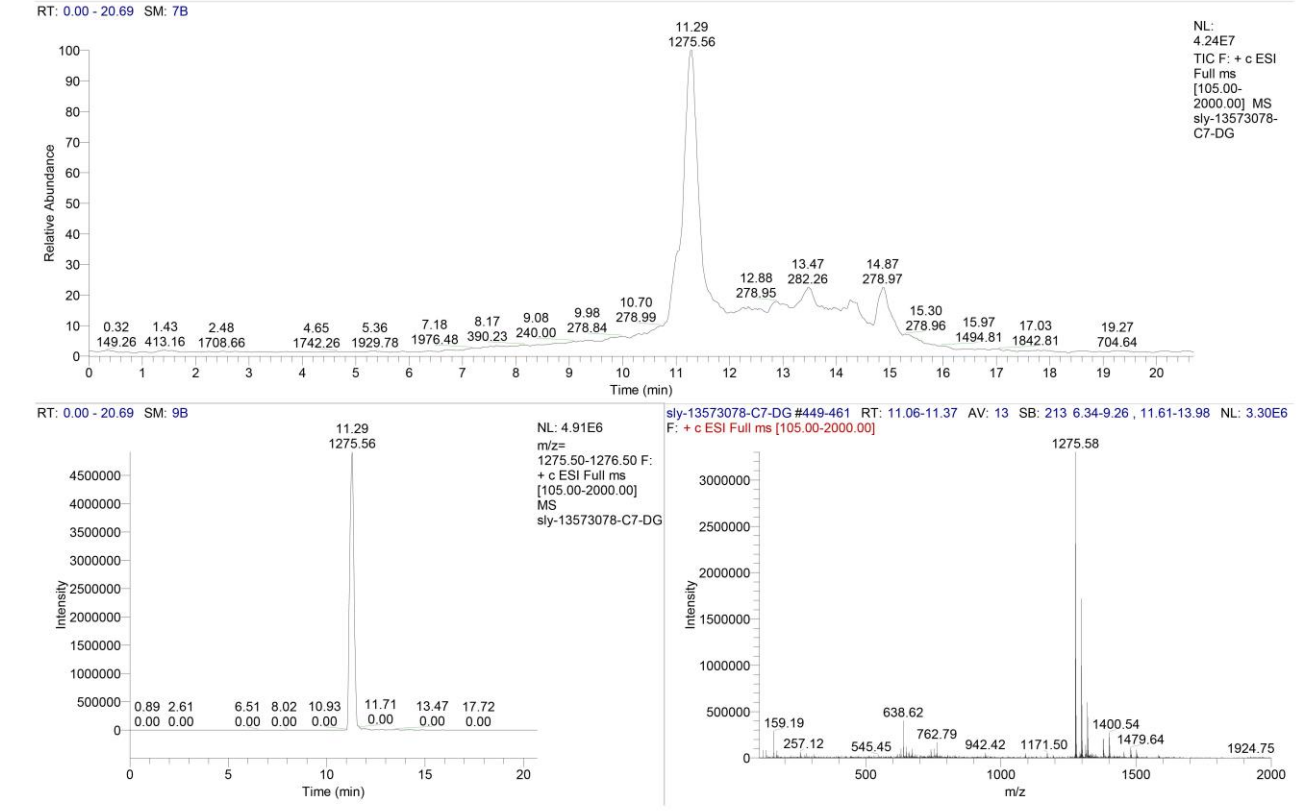


Figure S6. HPLC chromatogram and LC-MS spectrum of the cyclic peptide CYC7.

产品名称：
Peptide Name: Custom Peptide

订单编号：
Purchase order number: DH226015

批号：
Lot Number: B-06-1005

多肽序列：
Sequence: H-Cys-Ala-Asp-Thr-Asn-Trp-Leu-Val-Cys-OH（Cys&Cys bridge）

分子量：
Molecular Weight (daltons): 1022.17

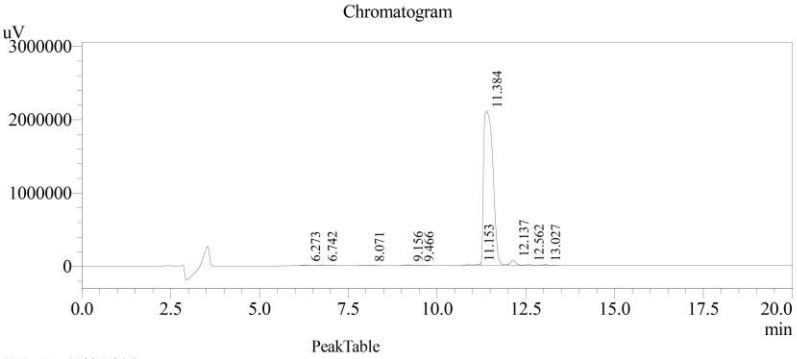
纯度：
Final Purity (HPLC %): 96.5%

产品重量：
Amount Supplied: 4 mg

产品外观： 白色冻干粉
Appearance： White lyophilized powder

储存条件： 长期储存 Long Term: -20℃
Storage Conditions： 短期储存 Short Term: 4℃

Chromatogram

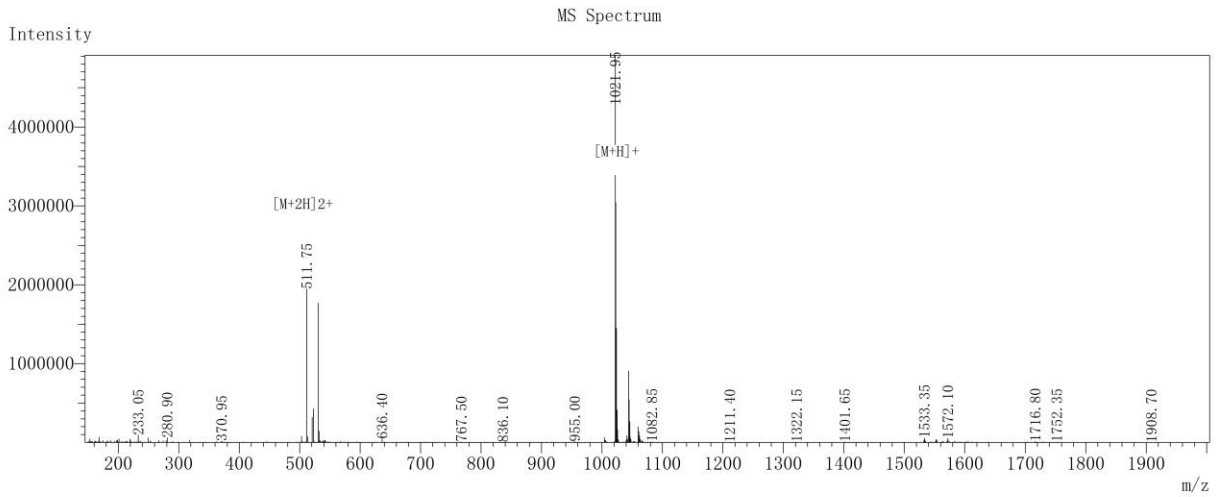


PeakTable

Detector A Ch1 214nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	6.273	78470	7265	0.197	0.327
2	6.742	61991	7093	0.156	0.319
3	8.071	50498	4607	0.127	0.207
4	9.156	54310	5946	0.137	0.267
5	9.466	24090	2487	0.061	0.112
6	11.153	178661	10682	0.449	0.480
7	11.384	38383568	2098752	96.570	94.339
8	12.137	738204	71198	1.857	3.200
9	12.562	91685	8386	0.231	0.377
10	13.027	85431	8272	0.215	0.372
Total		39746908	2224690	100.000	100.000

MS Spectrum

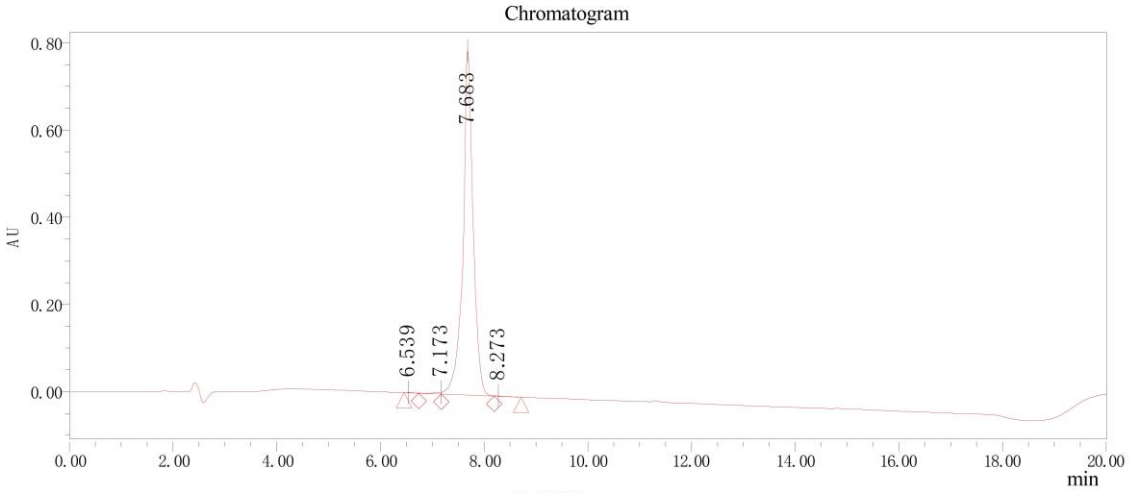


Sample Information
Dissolution method :0.1mg sample dissolved to 50%ACN and 50%H2O
Date Acquired :2022/07/11 12:08:17
Injection Volume :1ul
Name DH226015
Sequence :CADTNWLVC
Modification :C-C
Lot No. B-06-1005
Theoretical :1022.17
bserved

Interface :ESI
Nebulizing Gas Flow :1.50L
CDL Temp :250℃
CDL Volt
Block Temp :200

Prerod Bias :+4.5kv
Detector :-0.2kv
T.Flow :0.2ml/min
B.conc :50%H2O/50%MeOH

Figure S7. The analysis certification of the synthetic peptide DH226015.



PeakTable

	名称	保留时间 (分钟)	% 面积	面积 (微伏*秒)	高度 (微伏)
1		6.539	0.08	8271	991
2		7.173	0.40	43341	4174
3		7.683	99.41	10778724	790266
4		8.273	0.12	12626	1022

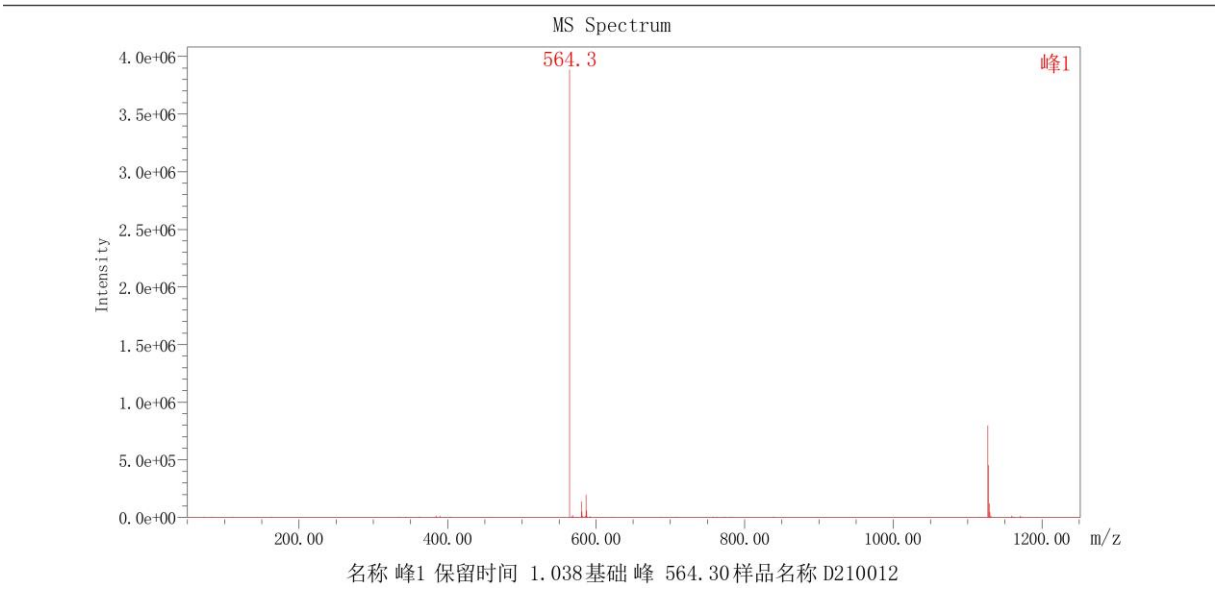


Figure S8. The analysis certification of the synthetic peptide D210012.

产品名称：
Peptide Name: Custom Peptide

订单编号：
Purchase order number: D210014

批号：
Lot Number: FM-02-0006

多肽序列：
Sequence: H-Cys-Ser-Gln-Ala-Trp-Trp-Tyr-Gln-Cys-OH（Cys&Cys bridge）

分子量：
Molecular Weight (daltons): 1172.3

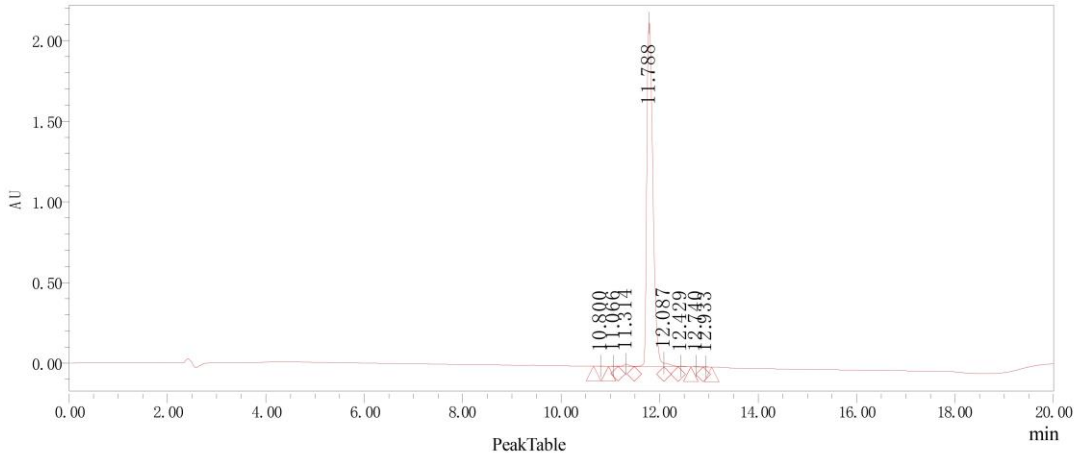
纯度：
Final Purity (HPLC %): 97.6%

产品重量：
Amount Supplied: 5 mg

产品外观： 白色冻干粉
Appearance： White lyophilized powder

储存条件： 长期储存 Long Term: -20℃
Storage Conditions： 短期储存 Short Term: 2℃~8℃

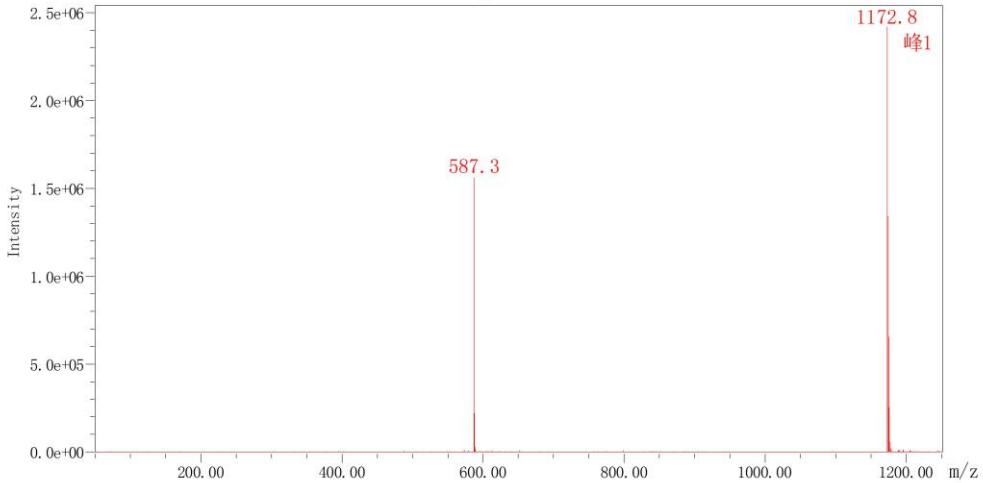
Chromatogram



PeakTable

	名称	保留时间（分钟）	% 面积	面积（微伏*秒）	高度（微伏）
1		10.800	0.05	9177	1166
2		11.066	0.10	18632	2786
3		11.314	0.60	113501	14223
4		11.788	97.60	18338575	2133439
5		12.087	1.13	212043	22559
6		12.429	0.21	39204	4744
7		12.740	0.22	42201	5523
8		12.933	0.09	16340	2891

MS Spectrum



名称 峰1 保留时间 2.736基础 峰 1172.76 样品名称 D210014

Figure S9. The analysis certification of the synthetic peptide D210014.

产品名称：
Peptide Name: Custom Peptide

订单编号：
Purchase order number: DH226016

批号：
Lot Number: B-06-1006

多肽序列：
Sequence: H-Cys-Phe-Leu-Asn-Val-Ser-Asn-Ala-Cys-OH（Cys&Cys bridge）

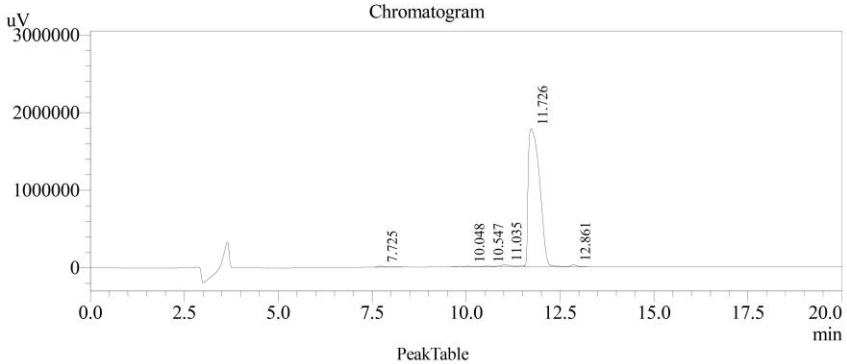
分子量：
Molecular Weight (daltons): 968.12

纯度：
Final Purity (HPLC %): 96.5%

产品重量：
Amount Supplied: 4 mg

产品外观： 白色冻干粉
Appearance： White lyophilized powder

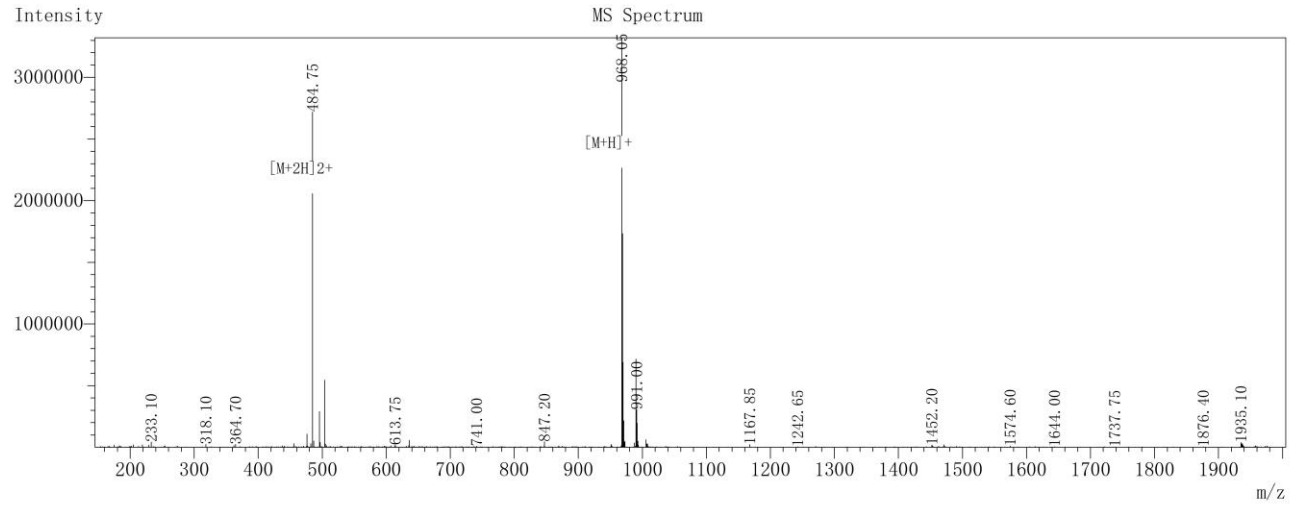
储存条件： 长期储存 Long Term: -20℃
Storage Conditions： 短期储存 Short Term: 4℃



PeakTable

Detector A Ch1 214nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	7.725	120552	13117	0.311	0.705
2	10.048	121709	6672	0.314	0.359
3	10.547	155555	8858	0.402	0.476
4	11.035	628262	25598	1.623	1.376
5	11.726	37382307	1779461	96.572	95.672
6	12.861	300689	26248	0.777	1.411
Total		38709075	1859956	100.000	100.000



Sample Informati
Dissolution method :0.1mg sample dissolved tc
50%ACN and 50%H2O
Date Acquired :2022/07/12 16:38:23
Injection Volume :1ul
Name DH226016
Sequence :CFLNVSNAC
Modification C-C:
Lot No. B-06-1006
Theoretical :968.125
bserved

Interface :ESI
Nebulizing Gas Flow :1.50L
CDL Temp :250℃
CDL Volt
Block Temp :200

Prerod Bias :+4
Detector :
T.Flow :0.2ml/min
B.conc :50%H2O/50%MeOH

Figure S10. The analysis certification of the synthetic peptide DH226016.

产品名称：
Peptide Name: Custom Peptide

订单编号：
Purchase order number: DH210901

批号：
Lot Number: A-09-1001

多肽序列：
Sequence: H-Cys-Gly-Ser-Leu-Asp-Trp-Pro-His-Cys-OH（Cys&Cys bridge）

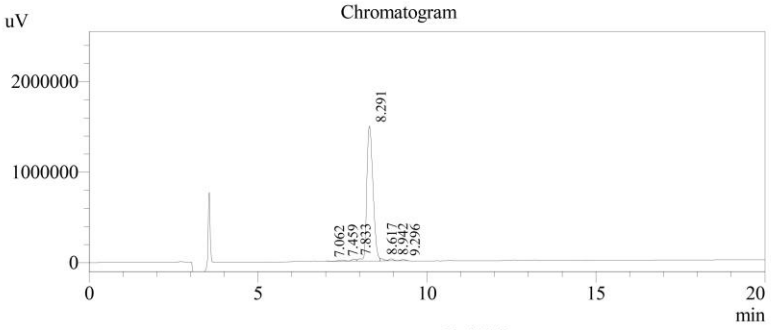
分子量：
Molecular Weight (daltons): 1015.14

纯度：
Final Purity (HPLC %): 95.4%

产品重量：
Amount Supplied: 5 mg

产品外观： 白色冻干粉
Appearance： White lyophilized powder

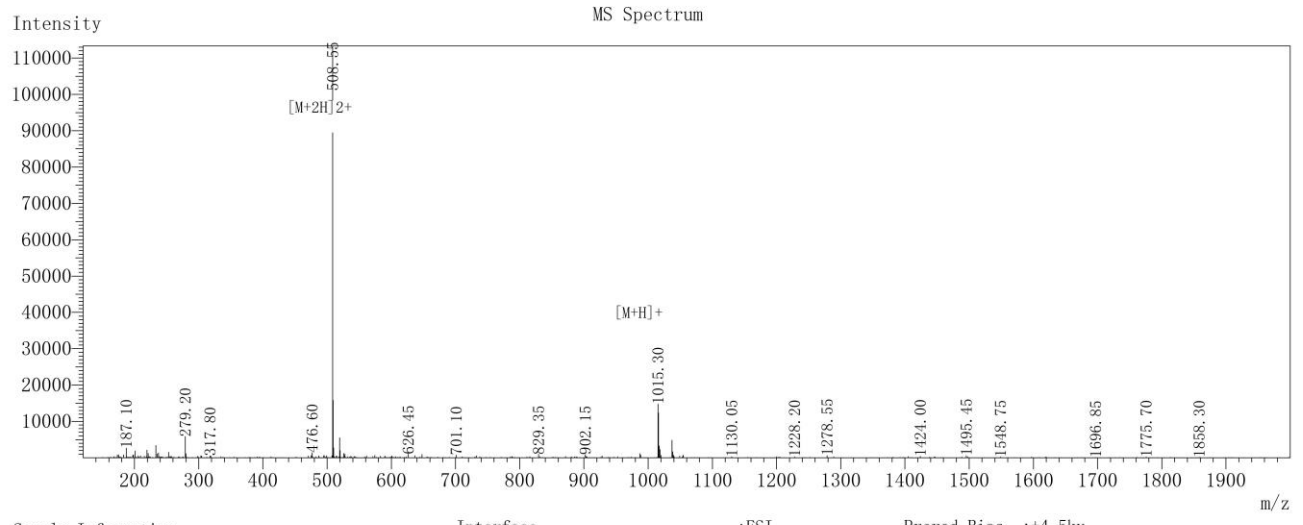
储存条件： 长期储存 Long Term: -20℃
Storage Conditions： 短期储存 Short Term: 4℃



PeakTable

Detector A Ch1 214nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	7.062	7656	1792	0.039	0.113
2	7.459	174838	12325	0.880	0.776
3	7.833	188577	21560	0.949	1.358
4	8.291	18978190	1490478	95.475	93.887
5	8.617	198911	26678	1.001	1.681
6	8.942	201543	21990	1.014	1.385
7	9.296	127843	12693	0.643	0.800
Total		19877557	1587516	100.000	100.000



Sample Information
Dissolution method :0.1mg sample dissolved to 100%H2O
Date Acquired :2021/10/12 11:22:03
Injection Volume :1ul

Interface :ESI
Nebulizing Gas Flow :1.50L
CDL Temp :250℃
CDL Volt
Block Temp :200

Prerod Bias :+4.5kv
Detector :-0.2kv
T.Flow :0.2ml/min
B.conc :50%H2O/50%MeOH

Name DH2109Q1
Sequence :CGSLDWPHC
Modification :C-C
Lot No. A-09-1001
Theoretical :1015.14
bserved

Figure S11. The analysis certification of the synthetic peptide DH210901.

产品名称：
Peptide Name: Custom Peptide

订单编号：
Purchase order number: DH226027

批号：
Lot Number: B-08-1009

多肽序列：
Sequence: H-Cys-Asn-Ser-Leu-Asn-Trp-Phe-Trp-Cys-OH（Cys&Cys bridge）

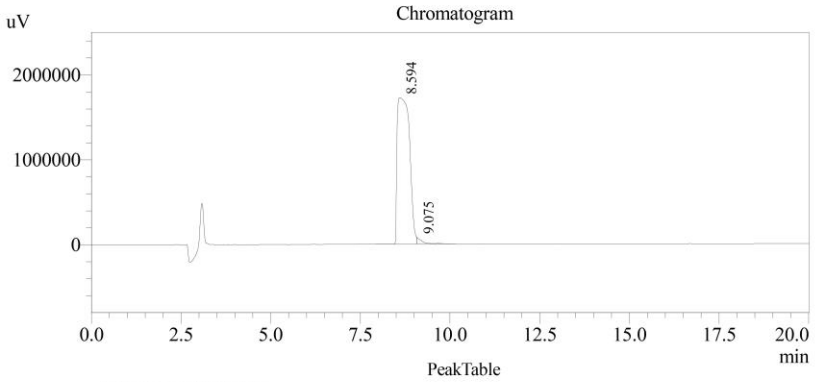
分子量：
Molecular Weight (daltons): 1170.33

纯度：
Final Purity (HPLC %): 98.0%

产品重量：
Amount Supplied: 4 mg

产品外观： 白色冻干粉
Appearance： White lyophilized powder

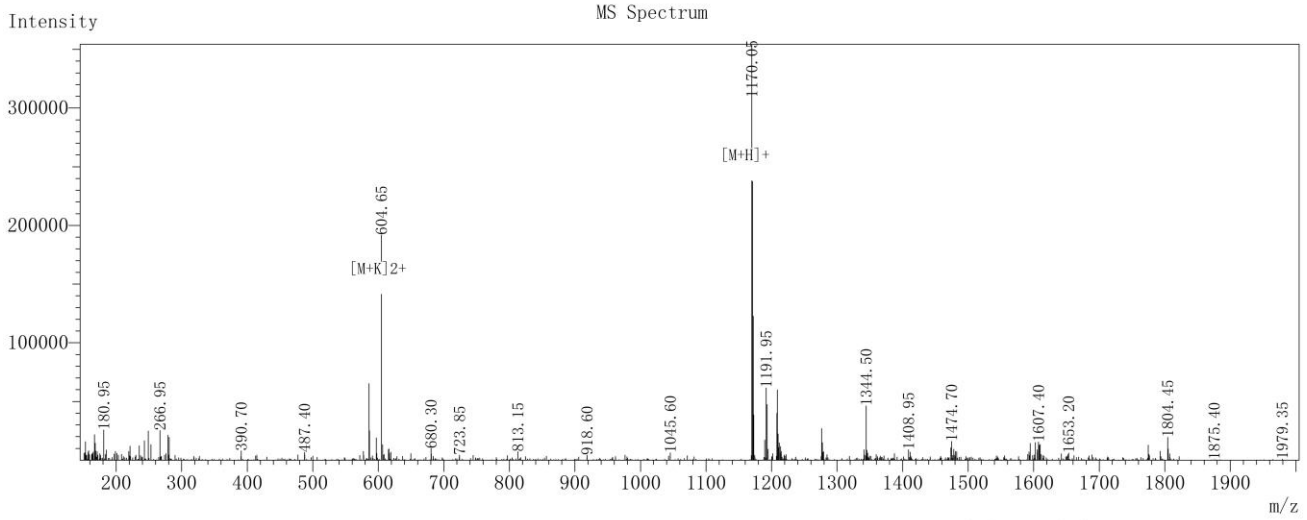
储存条件： 长期储存 Long Term: -20℃
Storage Conditions： 短期储存 Short Term: 4℃



PeakTable

Detector A Ch1 214nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	8.594	41260202	1723087	98.077	95.775
2	9.075	808972	76020	1.923	4.225
Total		42069174	1799107	100.000	100.000



Sample Information
Dissolution method :0.1mg sample dissolved to 50%ACN and 50%H2O
Date Acquired :2022/08/04 09:44:11
Injection Volume :1ul
Name DH226027
Sequence :CNSLNWFWC
Modification :C-C
Lot No. B-08-1009
Theoretical :1170.339
bserved

Interface :ESI
Nebulizing Gas Flow :1.50L
CDL Temp :250℃
CDL Volt
Block Temp :200

Prerod Bias :+4.5kv
Detector :-0.2kv
T.Flow :0.2ml/min
B.conc :50%H2O/50%MeOH

Figure S12. The analysis certification of the synthetic peptide DH226027.

产品名称：
Peptide Name: Custom Peptide

订单编号：
Purchase order number: DH226027-L

批号：
Lot Number: A-11-1001

多肽序列：
Sequence: H-Cys-Gly-Ser-Leu-Asp-Trp-Pro-His-Cys-OH

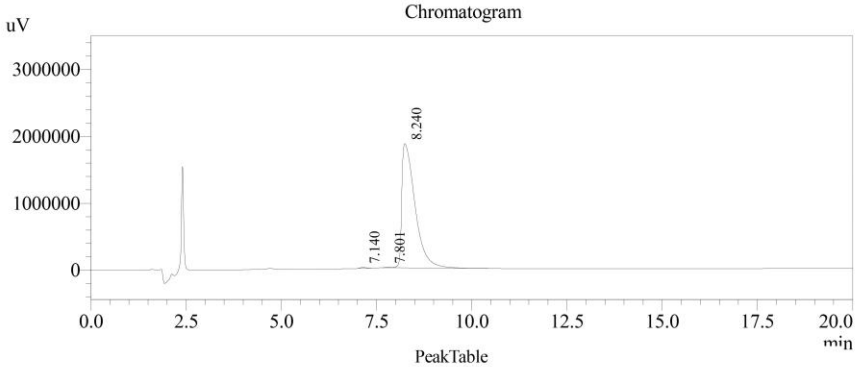
分子量：
Molecular Weight (daltons): 1017.14

纯度：
Final Purity (HPLC %): 99.4%

产品重量：
Amount Supplied: 100 mg

产品外观： 白色冻干粉
Appearance： White lyophilized powder

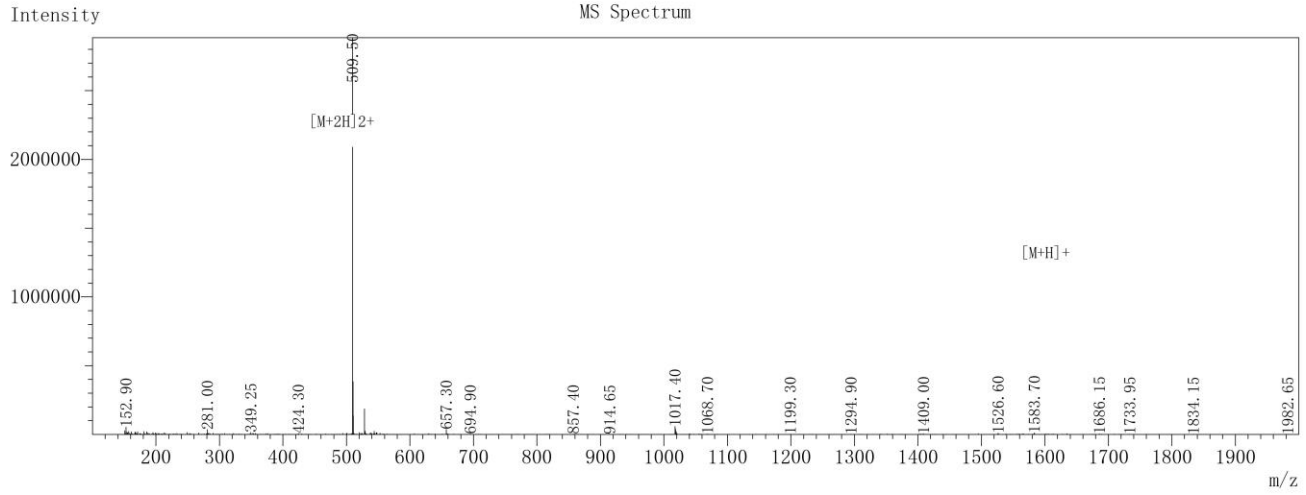
储存条件： 长期储存 Long Term: -20℃
Storage Conditions： 短期储存 Short Term: 4℃



PeakTable

Detector A Ch1 214nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	7.140	159618	14896	0.353	0.792
2	7.801	85460	7775	0.189	0.413
3	8.240	44915414	1857742	99.457	98.794
Total		45160492	1880413	100.000	100.000



Sample Informati
Dissolution method :0.1mg sample dissolved tc
100%H2O
Date Acquired :2021/11/11 14:41:06
Injection Volume :1ul

Interface :ESI
Nebulizing Gas Flow :1.50L
CDL Temp :250℃
CDL Volt
Block Temp :200

Prerod Bias :+4
Detector :
T.Flow :0.2ml/min
B.conc :50%H2O/50%MeOH

Name DH226027-L
Sequence :CGSLDWPHC
Modification :N/A
Lot No. A-11-1001
Theoretical :1017.14
bserved

Figure S13. The analysis certification of the synthetic peptide DH226027-L.