Supporting information

A siRNA-induced peptide co-assembly system as a peptide-based siRNA nanocarrier for cancer therapy

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Material

Abbreviations HCTU: 2-(1H-6-chlorobenzotriazol-1-yl)-1,1,3,3-tetramethyl uranium hexafluorophosphate; Fmoc： 9-fluorenylmethyloxycarbonyl; MNP: 2-Hydroxy-4'-(2-hydroxyethoxy)-2-methylpropiophenone； DIPEA: diisopropylethylamine; MAP: 4'-Methoxyacetophenone, RT: room temperature; HPLC: high-performance liquid chromatography; FBS: fetal bovine serum; PS: penicillin/streptomycin; DCM: dichloromethane; DMF: dimethylformamide; TFA: trifluoroacetic acid; Et2O: diethyl ether； TIS: triisopropylsilane; LC-MS: liquid chromatography–mass spectrometry. SPPS, solid-phase peptide synthesis.

Materials The MBHA resins and Fmoc-protected amino acid used for peptide synthesis by solid phase peptide synthesis (SPPS) and the agarose for gel retardation, mica for AFM, silicon for SEM and other chemical reagents were all purchased from commercial suppliers, such as GL Biochem (Shanghai) Ltd. J&K Co. Ltd., Huizhou Deep chemical technology co. LTD, Tianjin Damao Chemical Reagent Factory, Tianjin Yongda Chemical Reagent Company Limited or Shenzhen Tenglong Logistics Co. Commercial transfection reagent Lipo-2000 and Oligo were purchased from Thermo Fisher Scientific company. siRNA were all purchased from ShangHai Genepharma company.

Cell line and cell culture Human cervix cancer cell line, HeLa (ATCC® CCL-2™) were cultured in DMEM medium (Gibco) with addition of 10% (v/v) FBS (Gibco) and PS (100 μg/mL, Gibco). Human pancreatic cancer cell line, Miapaca-2 (CRL-1420, American Type Culture Collection), were cultured in DMEM medium (Gibco) with addition of 10% (v/v) FBS (Gibco), 2.5% (v/v) horse serum (Gibco) and PS (100 μg/mL, Gibco). Human ovary cancer cell line, PA-1 (ATCC® CRL-1572™) were cultured in MEM medium (Gibco) with addition of 10% (v/v) FBS (Gibco) and PS (100 μg/mL, Gibco). Human ovary cancer cell line, A2780 cell line was a generous gift from Dr. Fei Lu (School of Chemical Biology and Biotechnology, Peking University Shenzhen Graduate School, Shenzhen). A2780 was cultured in 1640 medium (Gibco) with addition of 10% (v/v) FBS (Gibco) and PS (100 μg/mL, Gibco). Liver-derived cell lines QSG-7701 (Institute of Cytology, Chinese Academy of Sciences, Shanghai, China) were cultured in DMEM medium (Gibco) with 10% (v/v) FBS (Gibco). Human kidney-derived cell lines HEK-293T (ATCC® CRL-11268™) were cultured in DMEM medium (Gibco) with addition of 10% (v/v) FBS (Gibco) and PS (100 μg/mL, Gibco). All the cell lines were cultured in Dulbecco's modified Eagle's (Gibco) dish at 37℃, 5% CO_2.

HPLC and Mass spectrometry All used peptides were purified by HPLC (SHIMAZU Prominence LC-20AT, UV detection at 220 or 254 nm) with a C18 analytic column (Agilent ZORBAX SB-Aq, 4.6 × 250 mm, 5 μm, flow rate 1.0 mL/min). Mobile phase was the in linear gradient mixtures of filtered ddH_2O with 0.1% TFA and pure acetonitrile. Peptides were analyzed by LC-MS (SHIMAZU LC-MS 8030, ESI-MS) with ESI positive ion mode.

Peptides preparation

Synthesis of unnatural amino acids S_5. The synthesis of unnatural amino acids S_5 is based on the former researches.

Synthesis of nonapeptides. The preparation of peptides is based on the standard Fmoc-based solid phase peptide synthesis (SPPS), the protocol of the SPPS is summarized by literatures showed below. MBHA resin (loading capacity: 0.37 mmol/g) were swelled with NMP for 30 min. Then the 50% (vol/vol) morpholine in DMF was used to deprotect the -Fmoc group from MBHA resin for 30min × 2. After washing with DCM and DMF for 3 times, amino acid coupling mixture (the Fmoc-protected amino acids (5.0 equiv), HCTU (4.9 equiv), DIPEA (10.0 equiv)), which is dissolved in DMF was added for coupling for 2.5h, followed by washing with DCM and DMF for 3 times. And after that add 50% (vol/vol) morpholine in DMF to deprotect the Fmoc group for the later amino acid coupling.

After coupled nine amino acids on MBHA resins, the nonapeptides were already synthesized and waiting for further purification. The resins were treated with a mixture of TFA/H_2O/TIS (95/2.5/2.5) for 2 h and dried by blowing nitrogen. After precipitated with Hexane/Et_2O (1:1 in volume) at 4°C, the mixture was further dissolved by 42% (vol/vol) acetonitrile/ water and purified by HPLC with UV detection at 220 nm or 280nm and later identified by LC-MS.

For the S-alkylation modification at Met on peptide, peptides were dissolved in 1% HCOOH solution. Then 3-4mg halohydrocarbon with different alkylation were added in solution and put on shaker for 12h at room temperature. Then this mixture was directly purified by HPLC analysis to get the S-alkylation products and identified by LC-MS. The LC-MS identifications of each screened peptide was attached at the end of supporting information.

Experimental section

S-alkylated peptide preparation. Peptide was synthesized by standard Fmoc-based solid phase peptide synthesis (SPPS) and purified by HPLC and identified by LC-MS. Took 2mg purified peptide and dissolved with 3ml ddH_2O. Then added 5mg alkylation reagent (for 1,2-xylene alkylation, we used 1,2-Bis(bromomethyl) benzene) and 30μl HCOOH and shaked for 18h at room temperature. After that we used the HPLC to seprate the S-alkylated peptide and use LC-MS to identified S-alkylated product.

Gel retardation. Screened peptide (dissolved in DEPC water) were qualified by its weight. As for peptide, whose sequence contain W amino acid, we used nanodrop to qualify its concentration in DEPC water at the absorption at 280nm. Screened peptides incubated with siRNA (0.4μg) in RNAase free tube at room temperature for 30 min, with a gradient concentration. Then use the 1% agarose gel 110V electrophoresis for 15min in TAE buffer solution with 40mM Tris-HCl, 1 % acetic acidv/v, and 1 mM ethylene diamine tetraacetic acid (EDTA). Golden View was used to stain the agarose gel to visualize the retardation of peptide-siRNA complex and further analyzed by a UV illuminator to compare the position of peptide-siRNA complex and free siRNA.

AFM (atomic-force microscopy). Peptide Wpc was incubated with siRNA (0.4μg) in RNAse free tube at room temperature for 10 min. Add ddH_2O to dilute, and then use pipettor to take 20μl to the surface of single mica for spin-coating. Finally, the peptide-siRNA co-assembly nanoparticles homogeneously on the surface of single mica were analyzed by AFM.

SEM (scanning electron microscopy). Peptide Wpc was incubated with siRNA (0.4μg) in RNAse free tube at room temperature for 10 min. Add ddH_2O to dilute, and use pipettor to take 30μl to the surface of silicon. After drying at 60^oC, the peptide-siRNA co-assembly nanoparticles on the surface of silicon were analyzed by SEM.

Flow cytometry analysis. HeLa cells were culture in DMEM medium with 10% FBS (v/v) in 37°C, 5% CO_2 incubator. HeLa cells (40% density) were washed with PBS and then incubate with Opti-MEM medium without FBS and PS for 1h. After 2.0μg $siRNA^{FAM}$ and 50μg peptide were slightly mixed for 10min, the FAM labelled siRNA-peptide co-assembly nanoparticles were slightly added in medium and mixed. Then HeLa cells were cultured at 37°C, 5% CO_2 for 3h. After washing with PBS for 3 times, trypsin digestion (1min), and re-suspend (500μl) in PBS, cellular fluorescence was analyzed using a BD FACSCalibur flow cytometer. The mean intracellular fluorescence intensity was analyzed by Flowjo 7.6.1 software. The values represent averages of three independent experiments.

Confocal microscopy image. HeLa cells (40% density) were washed with PBS for 2 times and later incubated with Opti-MEM medium without FBS and PS for 1h. After 2.0μg $siRNA^{FAM}$ and 50μg peptide were slightly mixed for 10min, the peptide-siRNA nanoparticles were slightly added in medium and further incubate at 37°C, 5% CO_2 for 3h. After washing with PBS for at least 3 times, cells were fixed by 4% formaldehyde (Alfa Aesar, MA) for 20min at room temperature. Then cells were washed with PBS for another 3 times for further stained by 1μg/ml 4', 6-diamidino-2-phenylindole (DAPI) (Invitrogen, CA) for 10mins. Finally cells were imaged by confocal microscopy.

siRNA transfection. HeLa cells, Miacapa-2 cells, PA-1 cells or A2780 cells (30% density) were washed with PBS for 3 times and the medium was replaced with Opti-MEM medium (500μl) without FBS and PS for 1h. After 2.0μg functional siRNA and 50μg peptide were slightly mixed for 10min, the co-assembly nanoparticles were added in medium and slightly mixed. After cultured at 37°C, 5% CO_2 for 5h, and 10% FBS, 1% PS were added in medium and continue cultured for 48h. (After 24h, 500μl fresh medium could be added for cell growth) Then washed with PBS for 2 times, extracted for RNA for later reverse transcription and real-time PCR analysis.

RNA extraction and Real-time PCR analysis. After transfected with peptide-siRNA nanoparticles for 48h, TRIzol reagent (Invitrogen) was used to extract the RNA from HeLa cells and a spectrophotometer (Nano-Drop ND-2000) was used to quantify the amount of extracted RNA. Then based on the amount of RNA, reverse transcription reagent was used to transcribe the mRNA to cDNA: total RNA (2μg) and commercial reverse transcriptase kit from Promega were mixed and react at 42°C for 1h according to the instruction. Finally, the fold change of mRNA level was analyzed by real-time PCR system with SYBR green dye according to the cDNA from reverse transcription.

Cell viability test. HeLa cells (30% density) were transfected with peptide-siRNA (Survivin) nanoparticles. 4h later, cells were imaged by Inverted microscope. And at 24h, cells are washed with PBS, digested with trypsin and re-cultured by fresh medium for another 24h and imaged by Inverted microscope to compare the anti-growth effect of peptide-siRNA nanoparticles.

Cytotoxicity test. MTT assay: 8000 Cells were cultured in 96-wells plate. After washed with PBS and changed

with fresh medium without FBS, different concentrations of samples were added in plate and incubate at 37°C, 5% CO_2 for 2h. Then add 10% FBS and 1% PS to continue culture cells for 24h. Then use CCK-8 kit to evaluate the cell viability by comparison of the absorption at 450nm.

Blood toxicity assay. Fresh mouse blood was collected from BALB/c mice, and add 10μl 10mg/ml heparin sodium immediately. Then 1ml of the whole blood was centrifuged at 1500rad for 10mins to isolate RBCs from blood and further washed with PBS for 2 times until the supernate was not red again. Then take 200μl fresh blood cells and diluted with 8ml PBS. To test the blood toxicity of peptide, different concentration of peptide (from 0.01μg/μl to 0.6μg/μl) was added in 0.5ml of diluted fresh blood cells (about $1x10^8$ cells/ml) respectively with 0.1% SDS as positive control. These mixtures were placed on a shaker with incubator at 37°C for 1.5h. Then after centrifugation, supernate of each sample was taken to measure its absorbance at 540nm and 655 nm (as a contrast) by an iMark microplate reader (BioRad, Hercules, CA)

Cell cycle assay. HeLa cells (30% density) were transfected with peptide-siRNA (Survivin) nanoparticles. After cultured at 37°C, 5% CO_2 for 5h, and 10% FBS, 1% PS were added in medium and continue cultured for 48h (at 24h, cells are washed with PBS, digested with trypsin and re-cultured by fresh medium). Then digested and stained with 70% ethanol at 4°C for 4h. After centrifugation and washed with PBS, cells were stained with mixture (20μl 10% Triton, 1μl (10mg/ml) RNase, 5μl (1mg/ml) PI and 200μl PBS) at 37°C for 30mins. Lastly, the cell cycle of HeLa cells transfected with nanoparticles was analyzed by flow cytomery.

Animal experimennts. Athymic nude mice (BALB/c ASlac-nu，4 weeks old) were obtained from Vital River Laboratory Animal Technology Co. Ltd. of Beijing, People's Republic of China and allowed an acclimation period of 1 week. Mice were maintained in an isolated biosafety facility for specific pathogen free (SPF) animals with bedding, food and water. All operations were carried out in accordance with the National Standard of Animal Care and Use Procedures at the Laboratory Animal Center of Peking University Shenzhen Graduate School, Guangdong Province, People's Republic of China (the permit number is IACUC-ER-0023-005).

***In vivo* mice imaging.** When the HeLa tumor volume on the mice reached approximately 200-300mm^3. Mice were intratumor injected with 80μl of peptide-$siRNA^{cy3}$ nanoparticles (2μg $siRNA^{cy3}$ + 50μg peptide). After anesthetized with isoflurane (5% isoflurane/1L O_2 concentration for the first time and 2–3% isoflurane/1L air concentration for maintenance), mice were immediately imaged to monitor the distribution of peptide-siRNA nanoparticles *in vivo* at each time point (10min, 1h, 4h, 8h, 12h and 24h). In this study, the imaging system is an ex/in vivo imaging system (CRI Maestro, USA) (ex: 704 nm; filter: 735 nm).

Preparation for paraffin section histological analysis (IHC). Organ tissues for histological analysis were all collected from the final day in mice experiment. Organ tissues were fixed in 4% formalin-saline at room temperature for 24h. Subsequently, tissues were embedded in paraffin blocks and 4mm thick paraffin sections were installed on glass slide for hematoxylin and eosin (H&E) staining and later examined by light microscopy (Olympus BX51). As for immunohistochemistry analysis, cervix carcinoma tissue sections were firstly immersed in 3% H_2O_2 for 5 minutes. Then 5% BSA was used to block the nonspecific binding sites of tissue for 15mins. Diluted antibody against Survivin was incubated with slides at 4°C overnight, followed by washing and incubating with Rabbit-probe HRP-polymer detection system, based on the instructions from supplier. Finally, slides were incubated with 3, 3'-diaminobenzidine substrate and counterstained with hematoxylin, according to the ImmPACT DAB Peroxidase Substrate Kit (Vector Laboratories) for 4min.

Supplementary figures

No.	Entry	Sequence	Modified group	Stabilized peptide	Loading ability*	N/P#
1	C	Fmoc-RRCES$_5$RCES$_5$-NH$_2$	N/A	No	>160μg	>78
2	R	Fmoc-RRMEHRMES$_5$-NH$_2$	N/A	No	40μg	20
3	R-Ph-c	Fmoc-RRMEHRMES$_5$-NH$_2$	1,2-xylene	Yes	40μg	56
4	F	Fmoc-RRMEHRMEF-NH$_2$	N/A	No	>20μg	>10
5	F-NH$_2$	NH$_2$-RRMEHRMEF-NH$_2$	N/A	No	>40μg	>24
6	F-allyl	Fmoc-RRMEHRMEF-NH$_2$	allyl	No	>40μg	>59
7	F-Me	Fmoc-RRMEHRMEF-NH$_2$	methyl	No	>40μg	>60
8	F-Ph	Fmoc-RRMEHRMEF-NH$_2$	phenyl	No	>40μg	>56
9	F-Ph-c	Fmoc-RRMEHRMEF-NH$_2$	1,2-xylene	Yes	>40μg	>57
10	W	Fmoc-RRMEHRMEW-NH$_2$	N/A	No	>40μg	>20
11	W-NH$_2$	NH$_2$-RRMEHRMEW-NH$_2$	N/A	No	>40μg	>23
12	W-allyl	Fmoc-RRMEHRMEW-NH$_2$	allyl	No	>40μg	>58
13	W-Me	Fmoc-RRMEHRMEW-NH$_2$	methyl	No	>40μg	>59
14	W-Ph	Fmoc-RRMEHRMEW-NH$_2$	phenyl	No	>40μg	>54
15	Wpc (W-ph-c)	Fmoc-RRMEHRMEW-NH$_2$	1,2-xylene	Yes	10μg	14
16	octadecane-W-1,2-xylene-(c)	octadecane-RRMEHRMEW-NH$_2$	1,2-xylene	Yes	>25μg	>34
17	octadec-9-ene-W-1,2-xylene-(c)	octadec-9-ene--RRMEHRMEW-NH$_2$	1,2-xylene	Yes	>25μg	>34
18	H	Fmoc-RRMEHRMEH-NH$_2$	N/A	No	>80μg	>.41

Tab. S1 Summary of screened peptide candidates. The siRNA loading ability of each peptide candidate was evaluated by agarose gel retardation (1%). C peptide was utilized as negative control without additional positive charges. To find the appropriate sequence, F/W/H/R peptides were prepared, F and W peptides were of higher siRNA loading capacity. As for peptides without further modifications on methionine, their modification groups were marked as N/A. *: the loading ability of each peptide is refer to 0.4μg siRNA. #: the N/P is the ratio of the nitrogen atoms on PEI to siRNA phosphate groups, for peptide siRNA carrier, we used the ratio of positive charges of peptide to the siRNA phosphates to describe the amount of peptide used for siRNA incorporation.

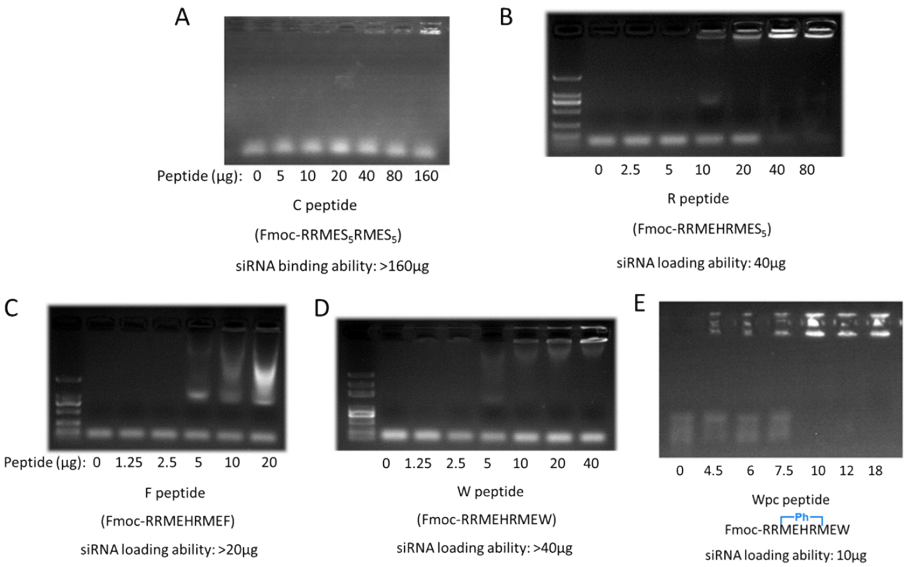


Fig. S1 Gel retardations of peptide 1, 2, 4, 10 and 15. (A) Gel retardation of R peptide (B-C) The substitution of unnatural amino acid to natural amino acid simplify the peptide preparation and showed high siRNA loading ability. (D) Wpc peptide with reducible crosslink showed highest siRNA loading ability that for 0.4μg siRNA.

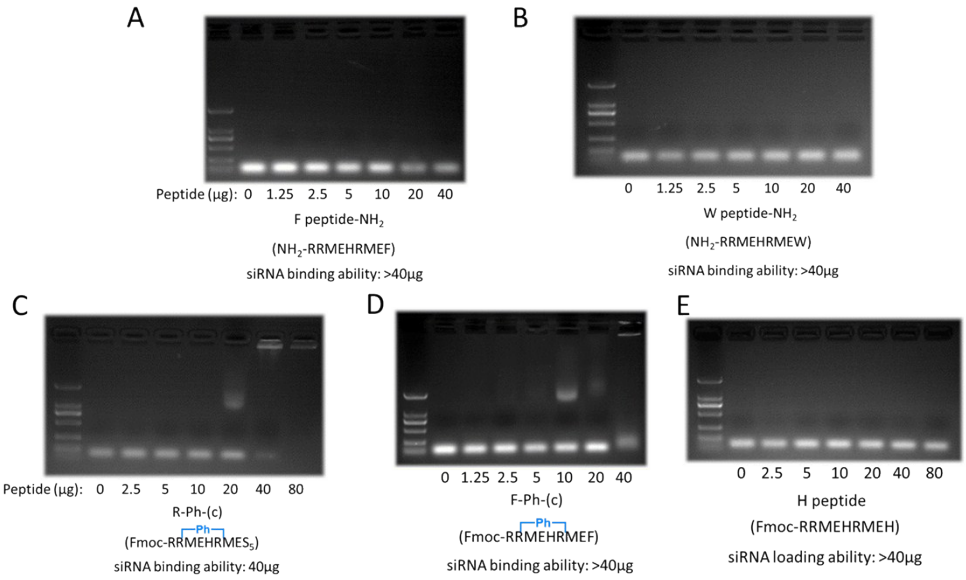


Fig. S2 Gel retardations of peptide 5, 11, 3, 9, 18. (A-B) For peptide 5 and 11, the siRNA loading ability of N terminal -NH_2 derivate peptides were greatly decreased. These results indicate that the lipophilicity of the terminal group is crucial for the incorporation of siRNA. (C-D) For peptide 3 and 9, their siRNA binding affinity is relative high. (E) Gel retardations of peptide 18 with low siRNA loading ability.

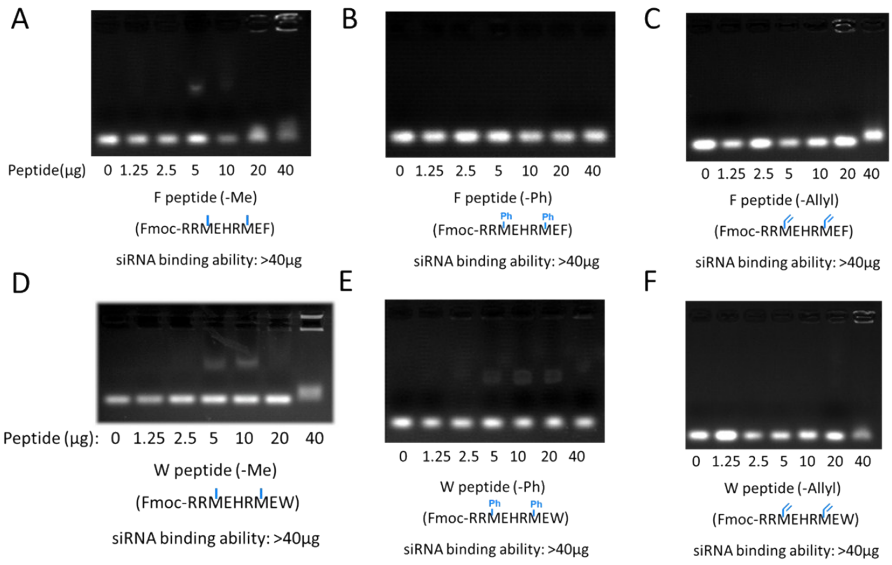


Fig. S3 Gel retardations of linear S-alkylation peptide 6, 7, 8, 12, 13, 14. (A-C) The gel retardations of peptides 6-8 with linear S-modification (allyl, methylation or phenyl at methionine), their siRNA binding affinity are significant decreased. (D-F) The gel retardations of peptides 12-14 with S-modification (allyl, methylation or phenyl at methionine), their siRNA loading affinity are relatively low.

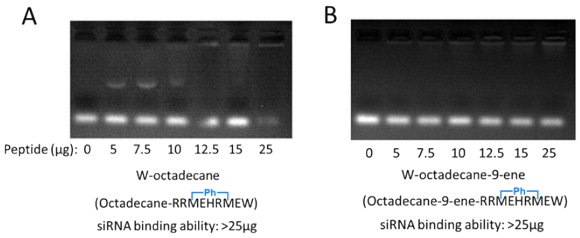


Fig. S4 Gel retardations of peptides 16-17 with N-terminal alkylation. (A) Gel retardation of peptide 16 with N-terminal octadecane substitution. Its siRNA binding affinity is high with about 25μg for 0.4μg siRNA. (B) Gel retardation of peptide 17 with N-terminal octadec-9-ene substitution. Its siRNA loading ability is significantly low.

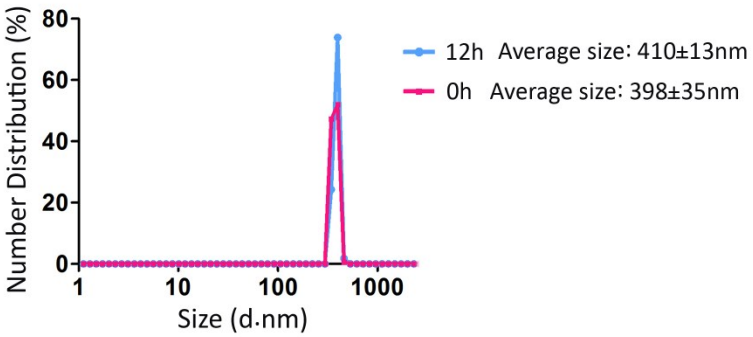


Fig. S5 DLS analysis of peptide-siRNA co-assembly nanoparticles incubated with PH=5.5 HCl 37°C for 12h. The particle size was stabilized in the PH=5.5 buffer incubation at 37℃ for 12h.

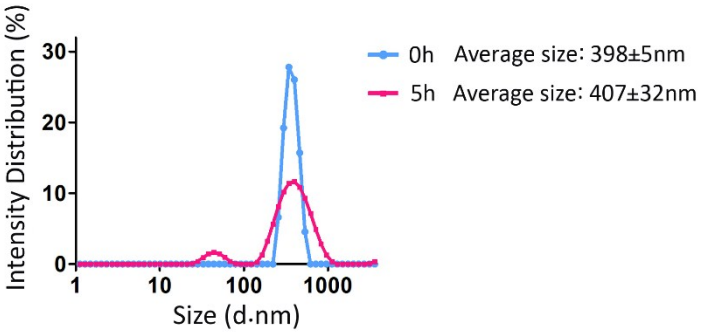


Fig. S6 DLS analysis of peptide-siRNA co-assembly nanoparticles incubated with 7.5% mouse serum at 37°C for 5h. The particle size was relative stabilized.

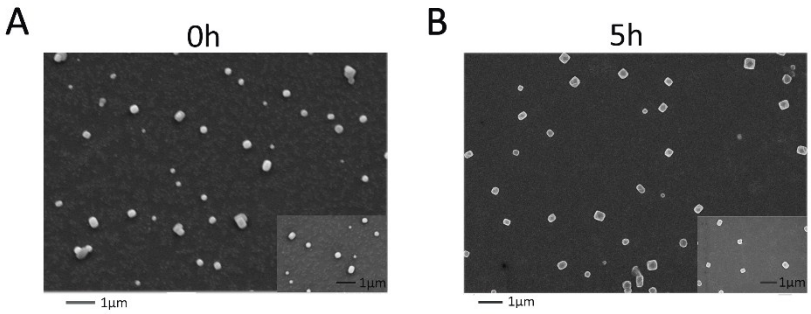


Fig. S7 SEM image of peptide-siRNA co-assembly nanoparticles incubated with 7.5% serum at 37°C for 5h. No

obvious change of co-assembly nanoparticles at 7.5% serum for 5h.

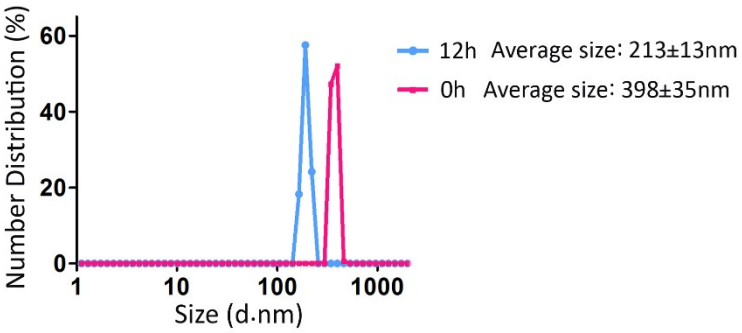


Fig. S8 DLS analysis of peptide-siRNA co-assembly nanoparticles incubated with 10mM GSH at 37°C for 12h. The particle size was significantly decreased.

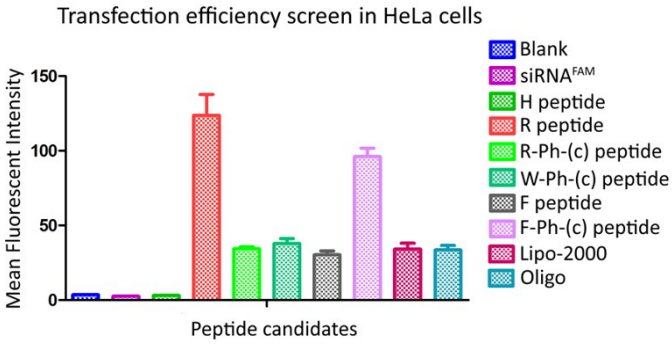


Fig. S9 Flow cytometry analysis of other screened peptides in HeLa cells. HeLa cells at 40% density were transfected with co-assembly nanoparticles (2.0μg siRNA^{FAM} and 50μg peptide) for 4h. After digestion and re-suspension, their transfection efficiency were analyzed by flow cytometry. Despite their relatively lower siRNA loading ability than peptide Wpc (W-ph-c), it is also possible that their cellular uptake are higher. So these flow cytometry analysis were conducted in HeLa cells. For R peptide, F peptide and F-Ph-(c), although their Mean fluorescence intensity are high, they have strong cytotoxicity to HeLa cells, which made them unsuitable for siRNA carrier. As for peptide R-Ph-(c), its fluorescent intensity is similar to peptide Wpc (W-ph-c) and commercial transfection reagent lipo-2000 and oligo. For H peptide, it cellular uptake is negligible like negative controls. It is also notable that for other screened peptides, as their siRNA loading ability are significantly lower than peptide Wpc (W-ph-c), so their cell penetration with siRNA cargo are not worth evaluating.

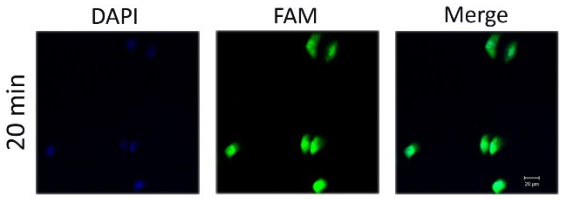


Fig. S10 Confocal microscopy image of HeLa cells transfected with peptide-siRNA co-assembly nanoparticles (2.0μg siRNA^{FAM} and 50μg peptide) for only 20 minutes. After transfected with nanoparticles for 20 minutes at 37°C, cells were washed with PBS and fixed with 4% formaldehyde. Then, stained by DAPI for confocal microscopy image. The homogeneously-distributed strong fluorescence signals proved the high transfection efficiency of Wpc (W-ph-c)

peptide.

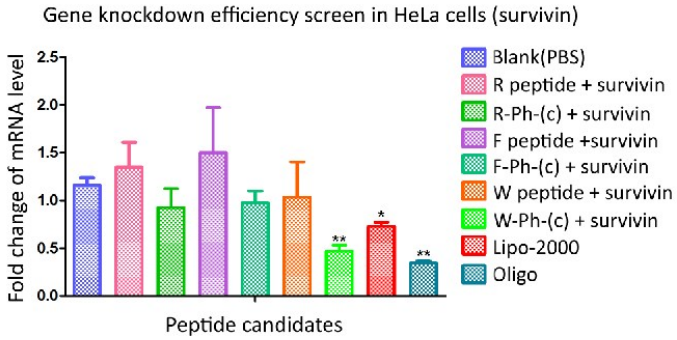


Fig. S11 RT-PCR analysis of screened peptides in HeLa cells with Survivin siRNA. As expected, peptide Wpc (W-ph-c) is the most efficient siRNA carrier in screened peptides, which showed a significant Survivin gene knockdown in HeLa cells.

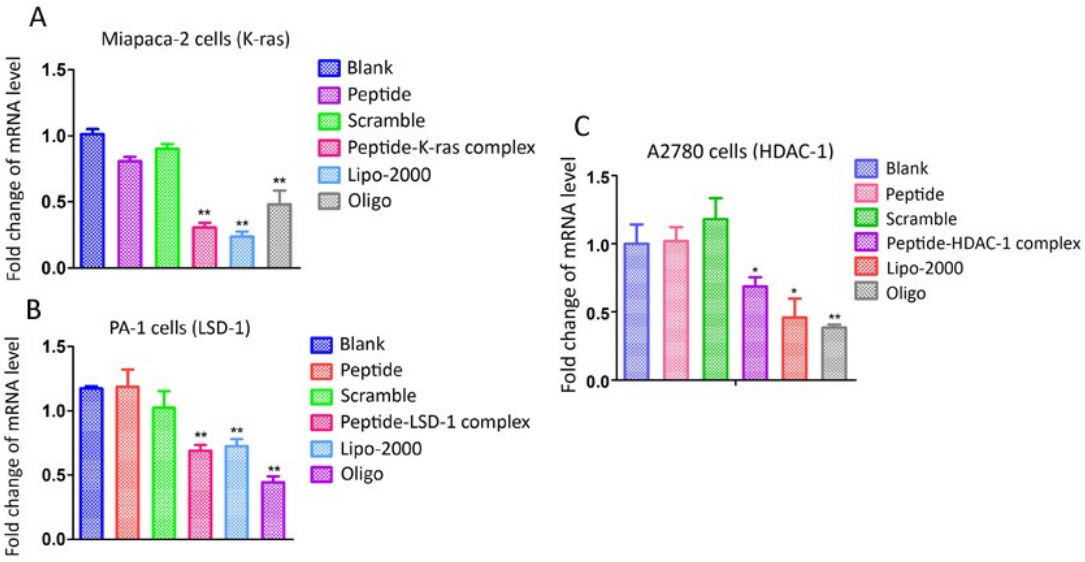


Fig. S12 RT-PCR analysis of Miacapa-2 cells, PA-1 cells and A2780 cells transfected with peptide-siRNA (K-ras for Miacapa-2 cells, LSD-1 for PA-1 cells and HDAC-1 for A2780 cells) co-assembly nanoparticles (2.0μg siRNA and 50μg peptide) for 48h. The mRNA level of K-ras, LSD-1 and HDAC-1 showed a clear knockdown in Miacapa-2 cells, PA-1 cells and A2780 cells. *, $P < 0.05$, **, $P < 0.01$ vs PBS (blank).

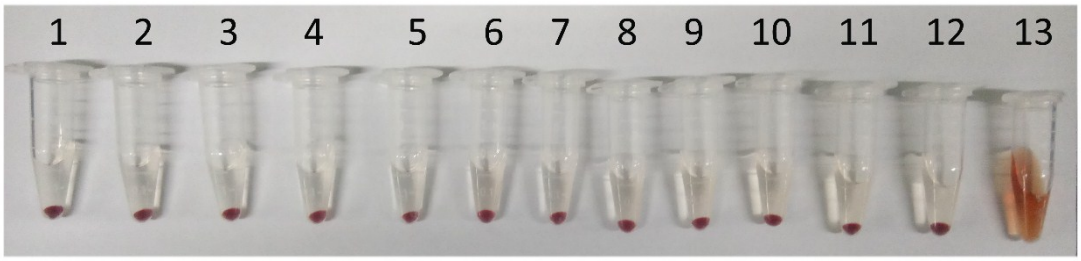


Fig. S13 Blood toxicity assay of Wpc (W-ph-c) peptide with fresh mouse blood. 10^7-10^8 blood cells were incubated with different concentration of Wpc peptide at 37°C for 1.5h. 1-12 are the Wpc peptide with different

concentrations (μg/μl): 0.01, 0.025, 0.05, 0.075, 0.1, 0.125, 0.15, 0.2, 0.3, 0.4, 0.5 and 0.6; 13 is 0.1% SDS as positive control.

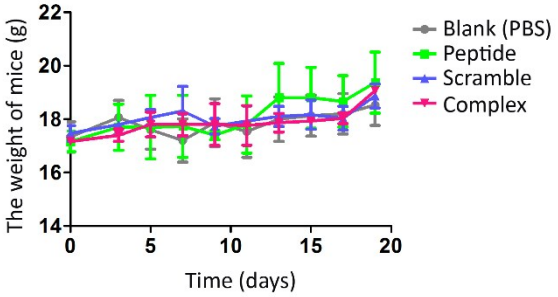


Fig. S14 The weight-time summary of mice in animal assay (from treated day 0 to day 19). There was no obvious weight loss of mice treated with peptide-siRNA nanoparticles during the examination period, indicating the low toxicity of our peptide-siRNA co-assembly nanoparticles.

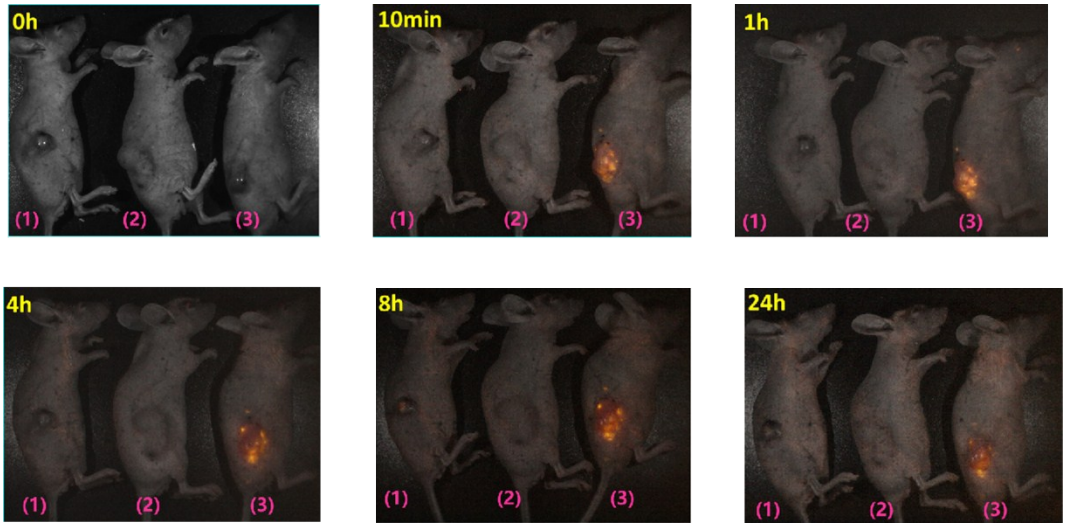


Fig. S15 The distribution of peptide-siRNA co-assembly nanoparticles. (1) PBS, (2) peptide (3) peptide-siRNA^{cy3} complex. The image of 0h is right before the injection of peptide-siRNA complex to show that the mice in this experiment have no fluorescent backgrounds. Mice were intratumor injected with peptide-siRNA^{cy3} co-assembly nanoparticles. At each time point: 10min, 1h, 4h, 8h and 24h, mice were anesthetized and imaged by IVIS Lumina II small animal in vivo optical imaging system. From the pictures showed above, peptide-siRNA^{cy3} nanoparticles were tightly gathered among the tumor section without diffusion even at 24h later. These results demonstrated that this novel peptide-siRNA co-assembly nanoparticle system is promising for *in vivo* anti-tumor growth gene therapy as it highly gathered among the tumor section.

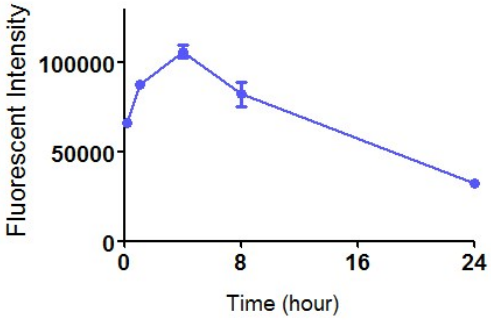


Fig. S16 Fluorescent intensity-time summary of peptide-siRNA^{cy3} nanoparticles (2.0μg siRNA^{FAM} and 50μg peptide) after intratumor injection. The fluorescent signal from tumor tissue still remained 40% after 24h.

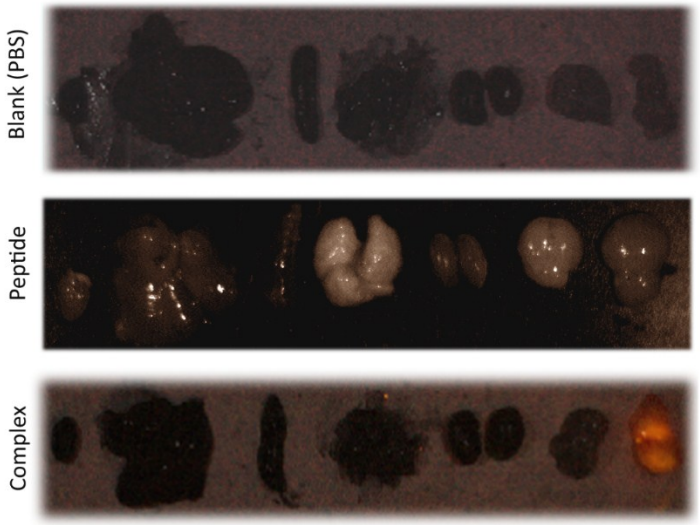


Fig. S17 The distribution of peptide-siRNA co-assembly nanoparticles in mice after intratumor injection for 24h. Complex: peptide-siRNA^{cy3} co-assembly nanoparticle. After 24h, the peptide-siRNA^{cy3} (2.0μg siRNA^{cy3} and 50μg peptide) were still mainly gathered at tumor section without any diffusion to other organs, including heart, liver, spleen, lung, kidney, brain.

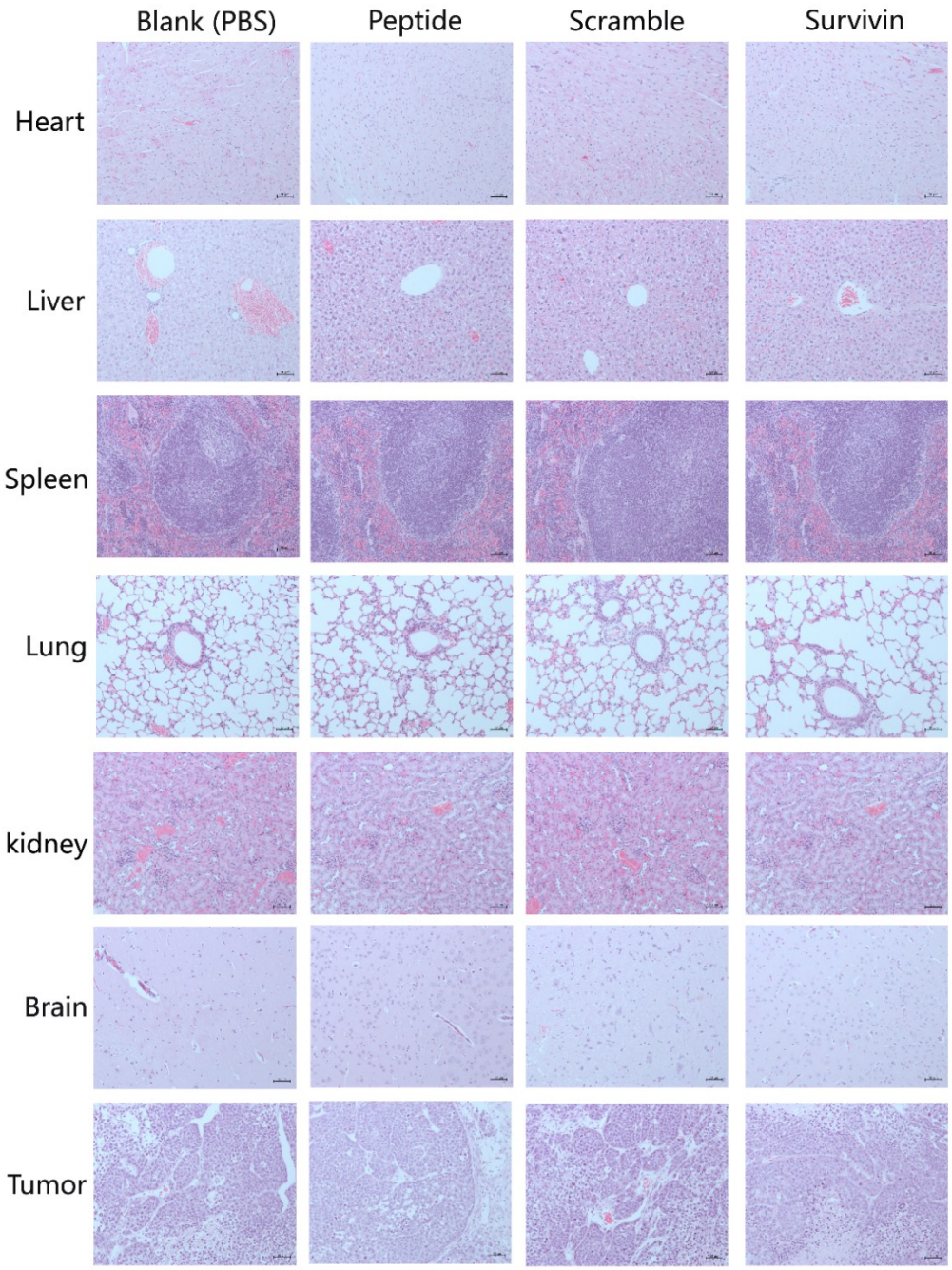


Fig. S18 H&E stained analysis of organs and tumor tissues collected from different groups of nude mice after 3 weeks treatment (0.32OD siRNA per mice, once every 2 days). Scramble: peptide-siRNA (Sramble) nanoparticles, Complex: peptide-siRNA (Survivin) nanoparticles. No obvious abnormity of peptide-siRNA (Survivin) nanoparticles than other controlled groups. MicroSpot Focusing Objective, 20X.

siRNA name	sequence (5'-3')
Human-LSD1 siRNA	AAGGAAAGCUAGAAGAAAATT
Human-HDAC1 siRNA	GCAAGCAGAUGCAGAGAUUTT
Human-Survivin siRNA	GGCUGGCUUCAUCCACUGCTT
Human-Bcl-2 siRNA	UGGAUGACUGAGUACCUGATT
Human-K-ras siRNA	GUUGGAGCUGAUGGCGUATT
Negative control	UUCUCCGAACGUGUCACGUTT

Tab. S2 Sequences of used siRNA.

Primer name	Orientation	Sequence (5'-3')
Human HDAC1	Forward	GGGATCGGTTAGGTTGCTTC
Human HDAC1	Reverse	TTGTCAGGGTCGTCTTCGTC
Human Kras	Forward	AGAGTGCCTTGACGATACAGC
Human Kras	Reverse	ACAAAGAAAGCCCTCCCCAGT
Human β-Actin	Forward	TCCAGCCTTCCTTCTTGGGTATG
Human β-Actin	Reverse	GAAGGTGGACAGTGAGGCCAGGAT
Human BCL2	Forward	GACTTCGCCGAGATGTCCAG
Human BCL2	Reverse	GGTGCCGGTTCAGGTACTCA
Human LSD1	Forward	AGCGTCATGGTCTTATCAA
Human LSD1	Reverse	GAAATGTGGCAACTCGTC
Human Survivin	Forward	TTGCGCTTTCCTTTCTGTCA
Human Survivin	Reverse	AATTTTCTTCTTGGCTCTTT

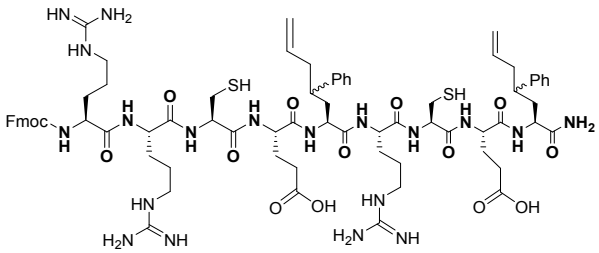
Tab. S3. Sequences of used primers.

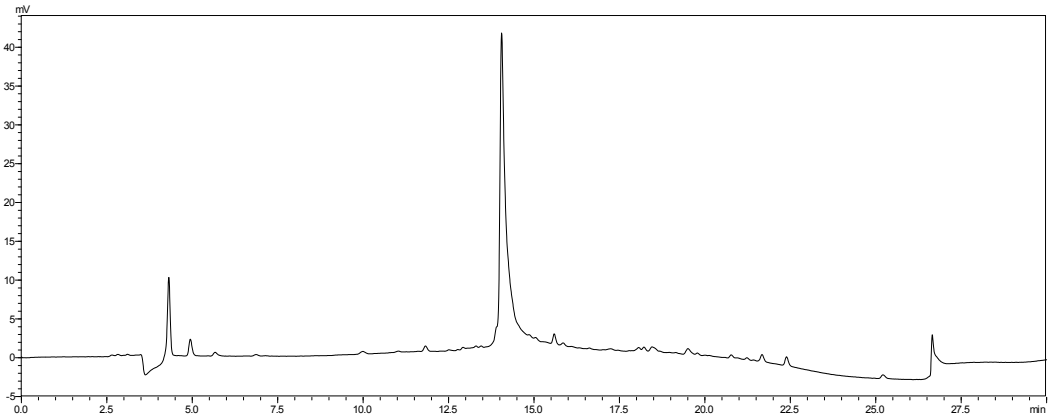
Calculated MS summary of screened peptides

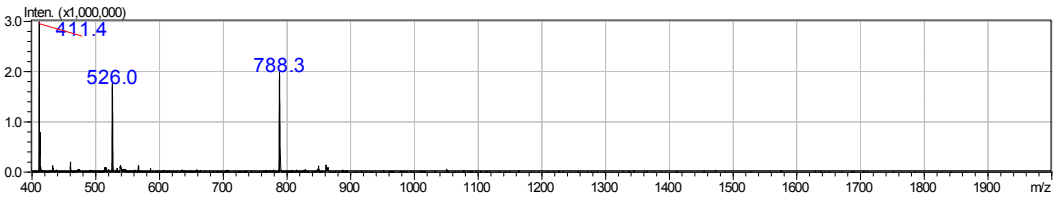
No.	Entry	Sequence	Calculated MS	Observed MS
1	C	Fmoc-RRCES$_5$RCES$_5$-NH$_2$	1576.7	526.0, 788.3
2	R	Fmoc-RRMEHRMES$_5$-NH$_2$	1565.7	523.3, 784.2
3	R-Ph-c	Fmoc-RRMEHRMES$_5$-NH$_2$ (Ph)	1670.8	418.3, 557.3
4	F	Fmoc-RRMEHRMEF-NH$_2$	1512.5	505.2, 757.2
5	F-NH$_2$	NH$_2$-RRMEHRMEF-NH$_2$	1289.7	430.9, 645.8
6	F-allyl	Fmoc-RRMEHRMEF-NH$_2$	1565.7	523.4
7	F-Me	Fmoc-RRMEHRMEF-NH$_2$	1544.8	514.6
8	F-Ph	Fmoc-RRMEHRMEF-NH$_2$ (Ph)	1667.9	557.3
9	F-Ph-c	Fmoc-RRMEHRMEF-NH$_2$ (Ph)	1616.7	539.3
10	W	Fmoc-RRMEHRMEW-NH$_2$	1550.7	518.2, 776.8
11	W-NH$_2$	NH$_2$-RRMEHRMEW-NH$_2$	1328.6	444.2, 665.5
12	W-allyl	Fmoc-RRMEHRMEW-NH$_2$	1604.9	536.0
13	W-Me	Fmoc-RRMEHRMEW-NH$_2$	1580.8	527.6
14	W-Ph	Fmoc-RRMEHRMEW-NH$_2$ (Ph)	1704.8	440.3, 568.6
15	Wpc (W-ph-c)	Fmoc-RRMEHRMEW-NH$_2$ (Ph)	1655.9	414.5, 552.3
16	octadecane-W-1,2-xylene-(c)	octadecane-RRMEHRMEW-NH$_2$ (Ph)	1695.8	425.1, 566.3
17	octadec-9-ene-W-1,2-xylene-(c)	octadec-9-ene--RRMEHRMEW-NH$_2$ (Ph)	1696.0	425.2, 566.4
18	H	Fmoc-RRMEHRMEH-NH$_2$	1502.1	501.9, 752.1

LC-MS spectrum of screened peptides

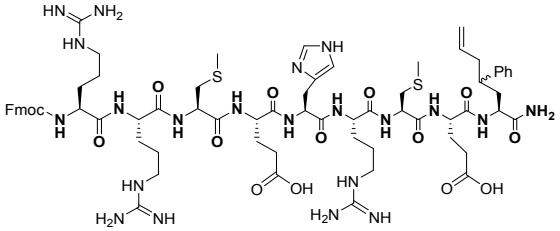
C peptide

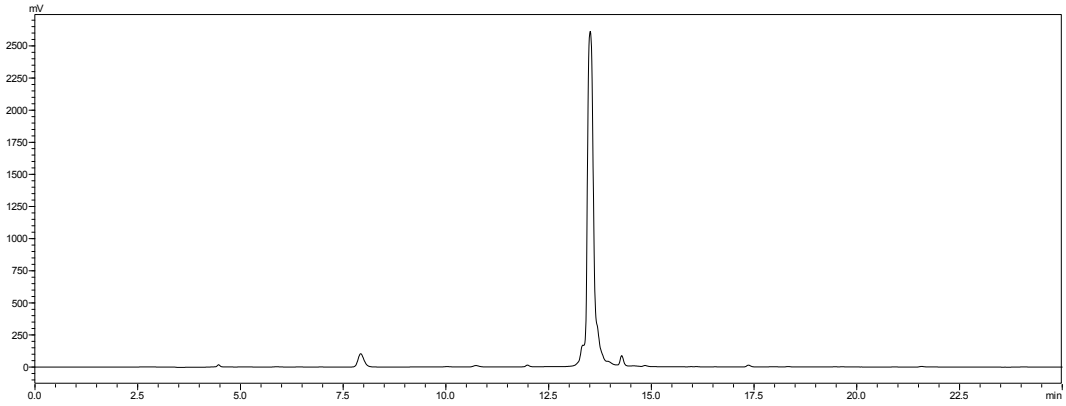


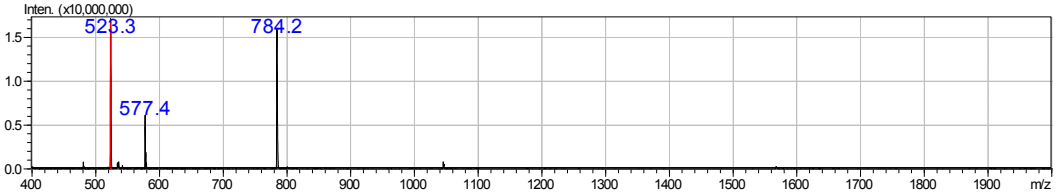




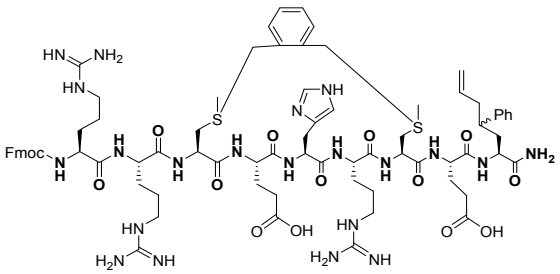
R peptide

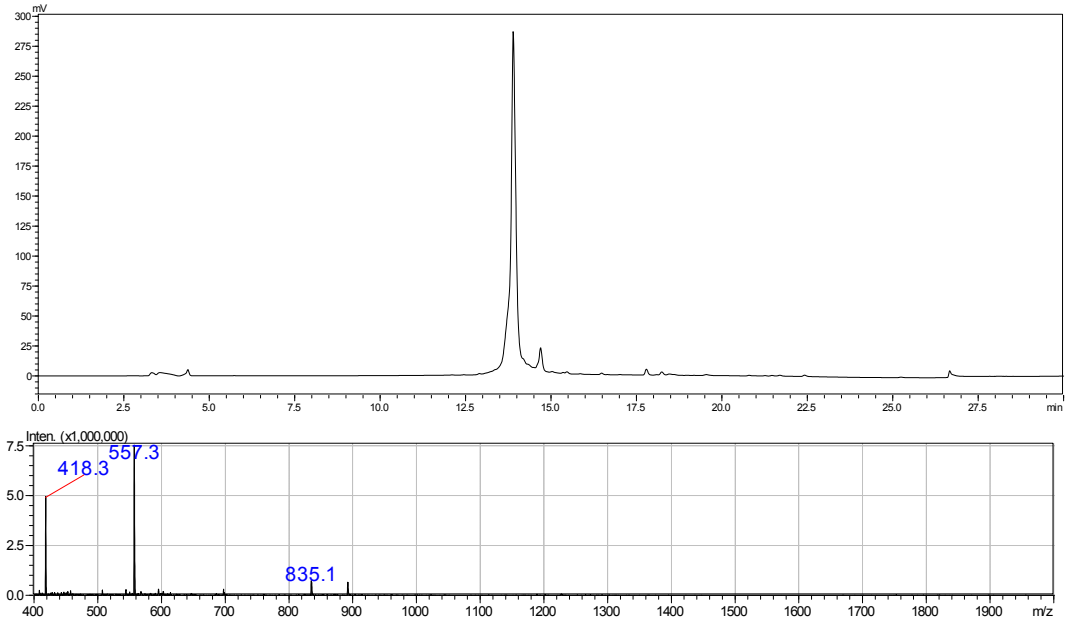




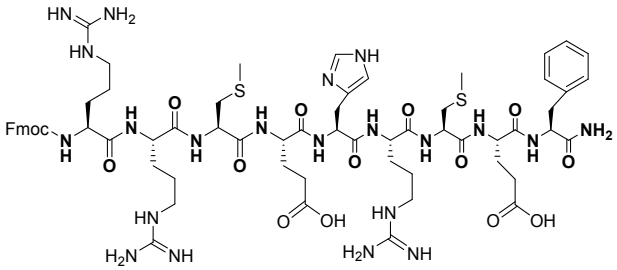


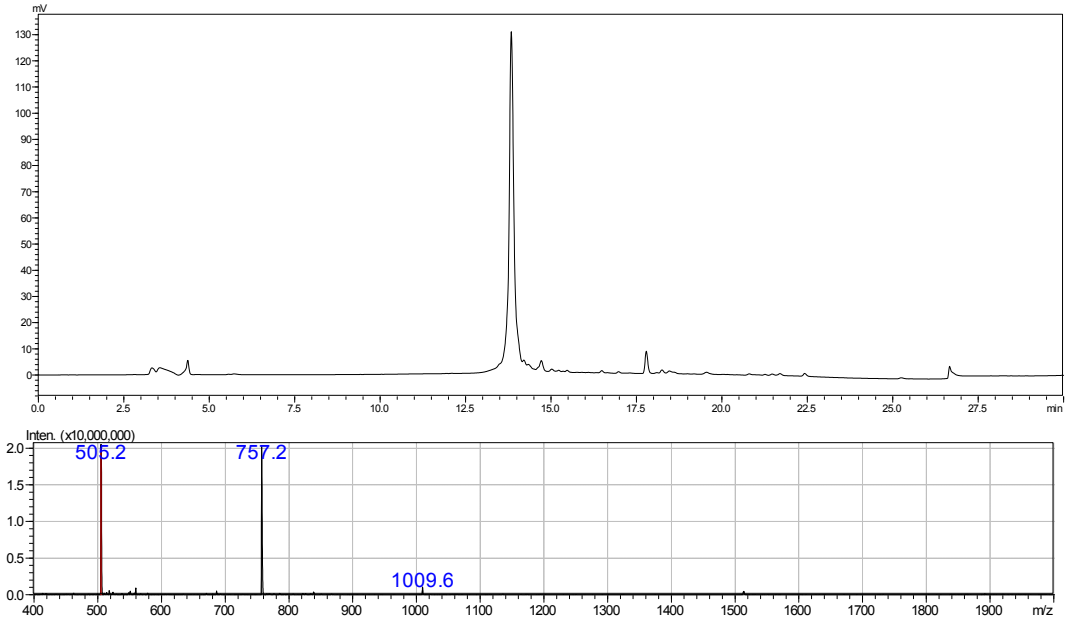
R-Ph-(c)



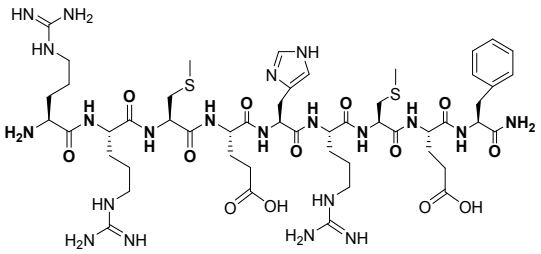


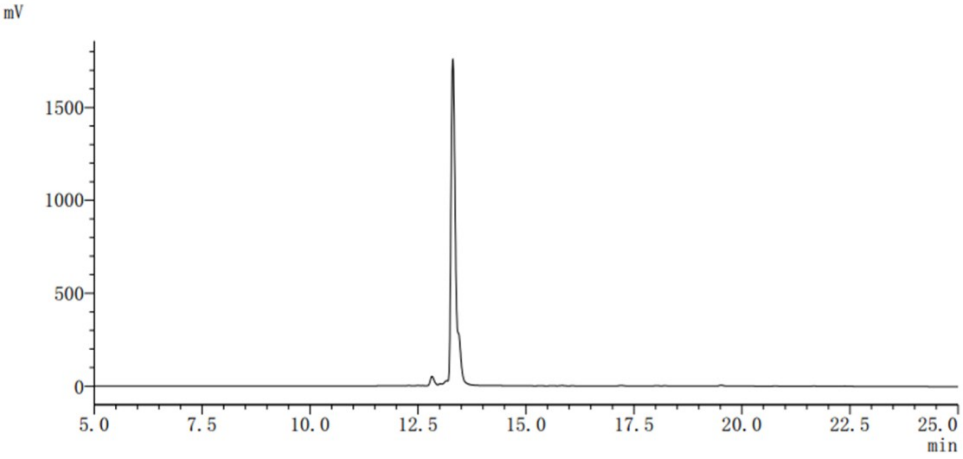
F peptide





F-NH_2

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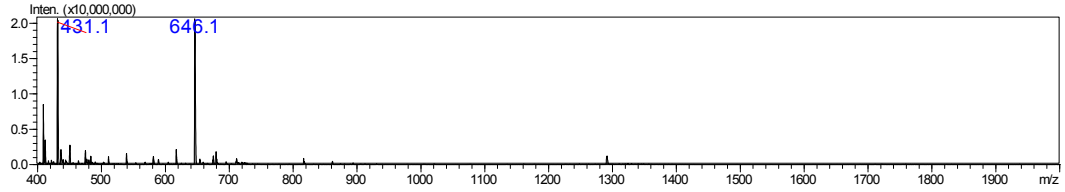
22.5
25.0
min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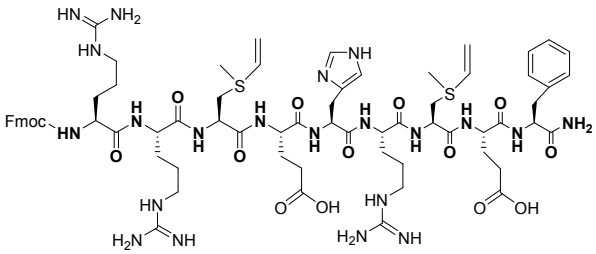
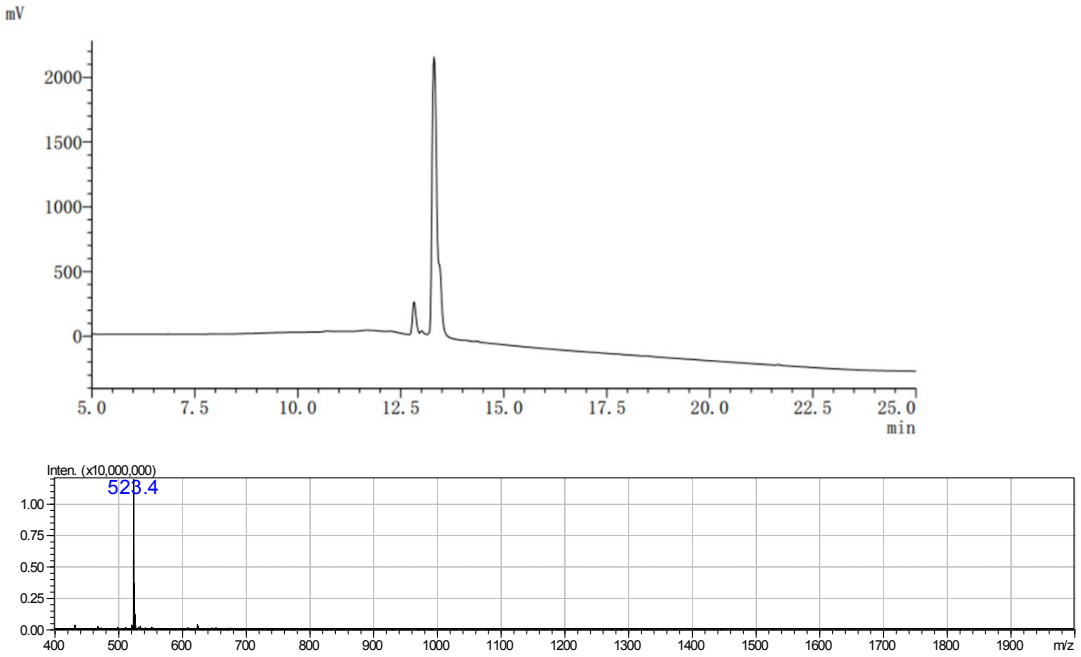
1700
1800
1900
m/z

F-allyl

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12.5
15.0
17.5
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22.5
25.0
min
Inten. (x10,000,000)
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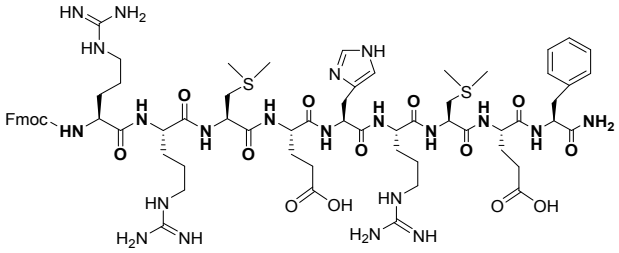
m/z

F-Me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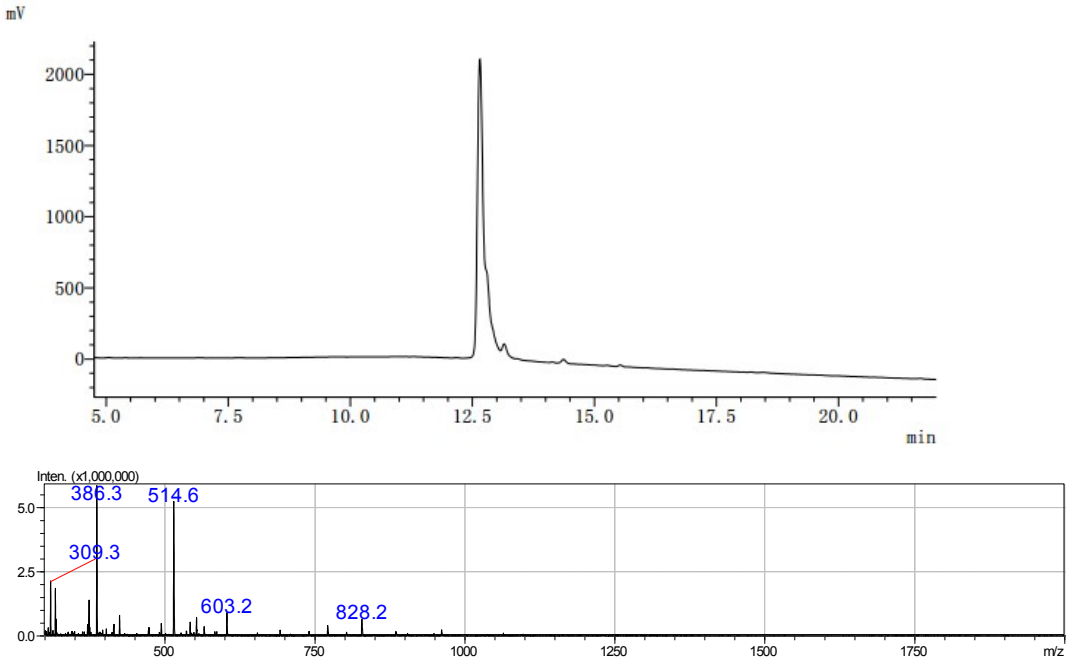
NH2
HN
Fmoc
HN
O
H
N
O
S
NH
N
OH
NH2
H2N
NH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1000
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5.0
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17.5
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min
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514.6
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1000
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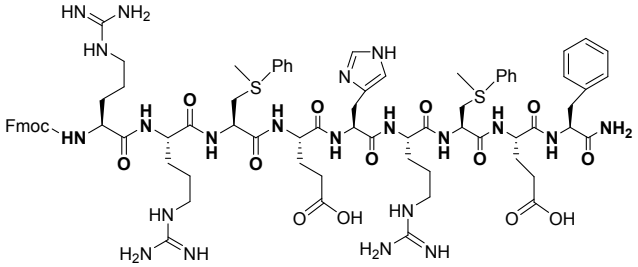
1500
1750
m/z

F-Ph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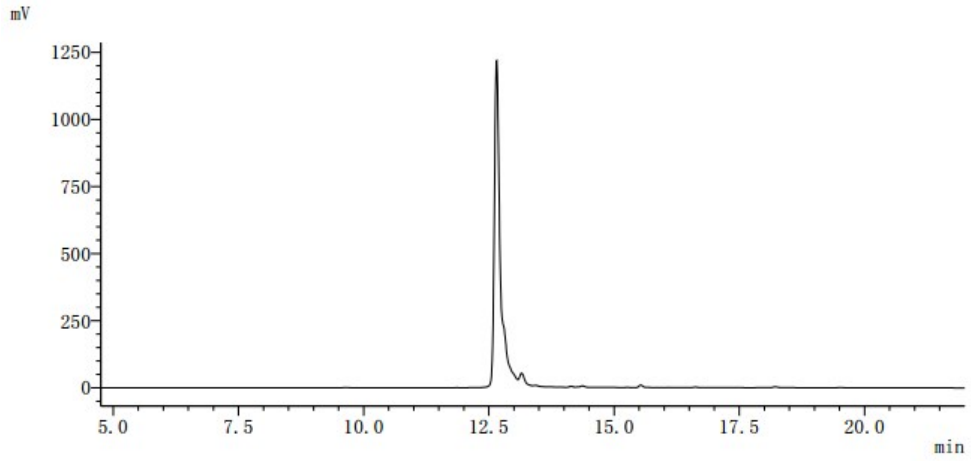
Ph
Ph
NH
NH2
OH
OH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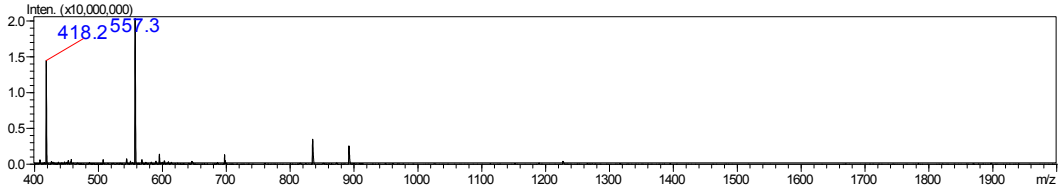
min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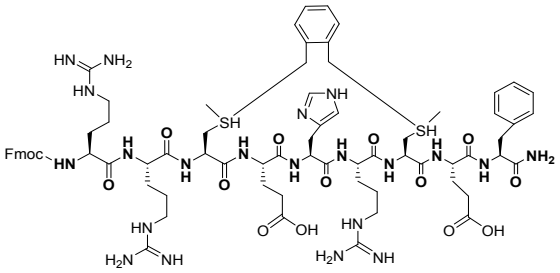
1600
1700
1800
1900
m/z

F-Ph-(c)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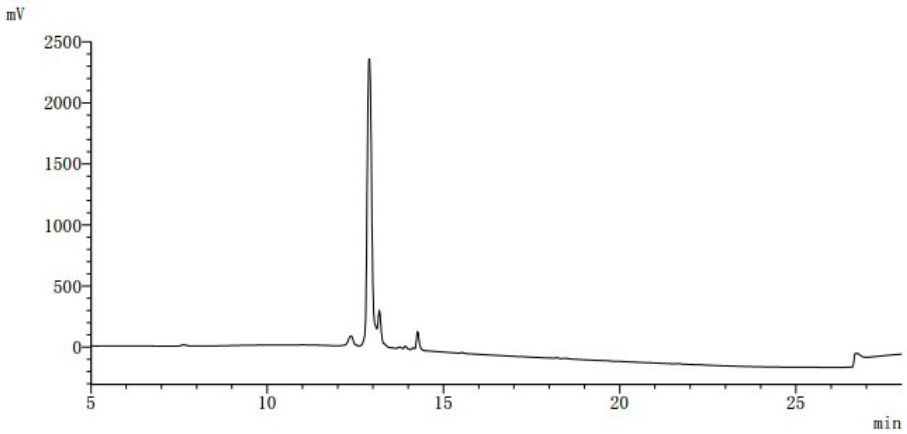
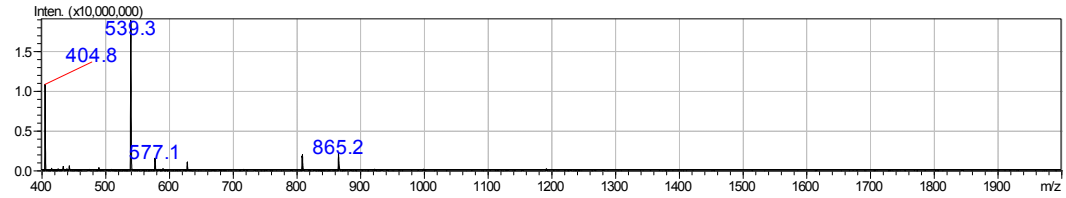
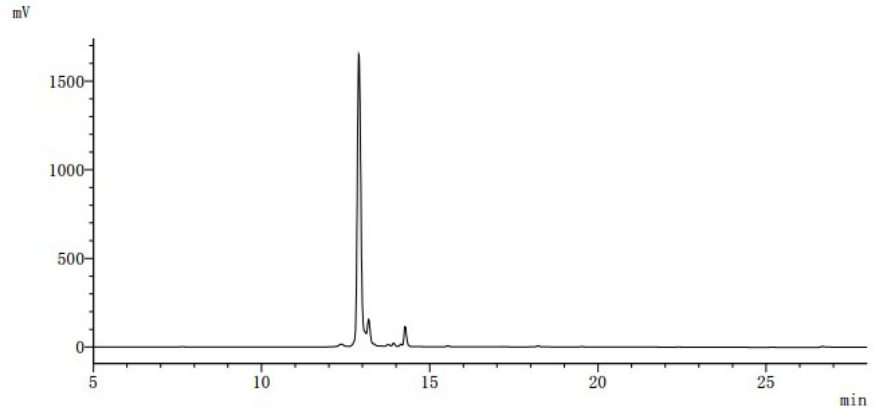
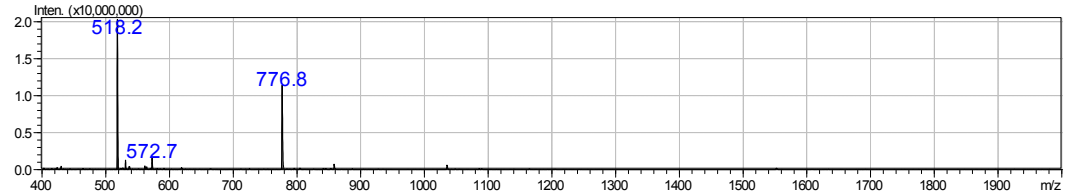
SH
NH
NH2
OH
OH



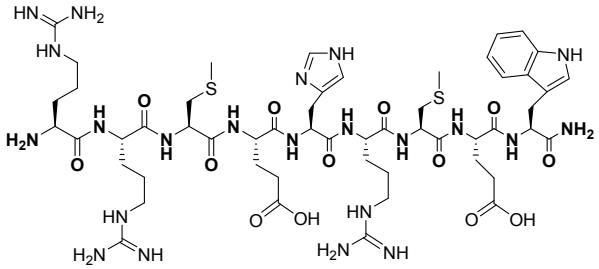


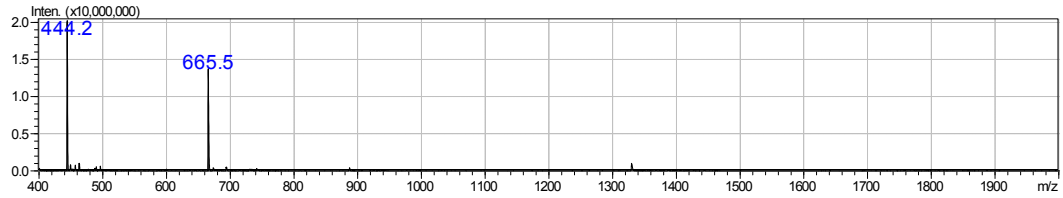
W peptide





W-NH_2

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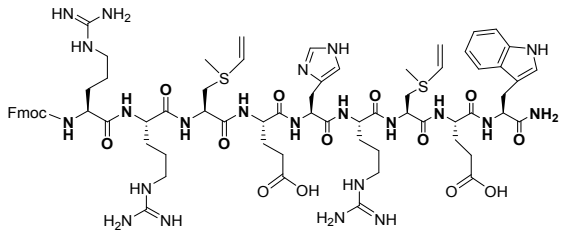
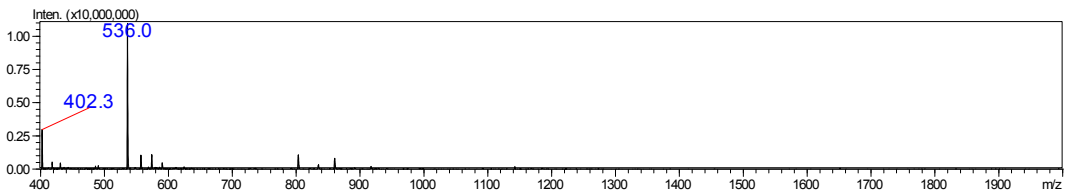
1000
1100
1200
1300
1400
1500
1600
1700
1800
1900
m/z

W-allyl


Fmoc
NH2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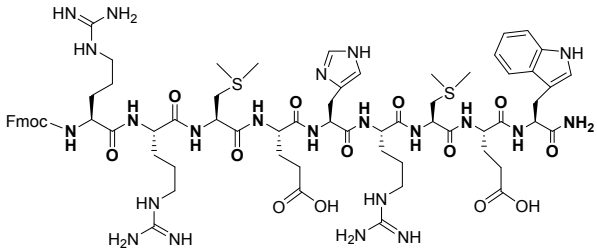
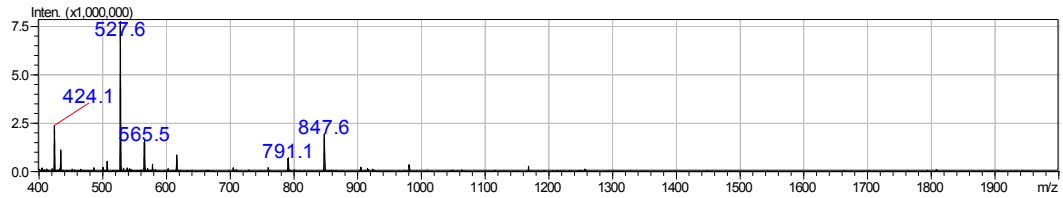
1400
1500
1600
1700
1800
1900
m/z

W-Me

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565.5
847.6
791.1
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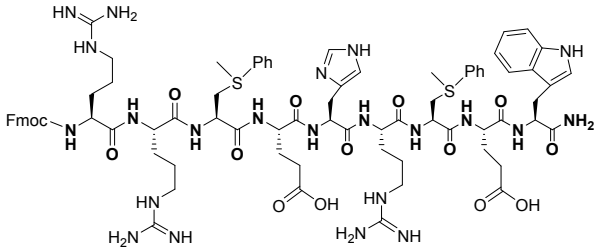
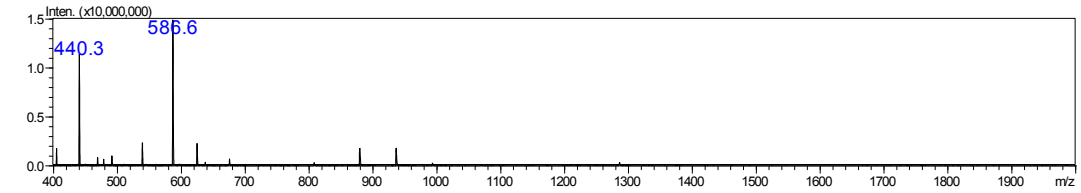
1400
1500
1600
1700
1800
1900
m/z

W-Ph





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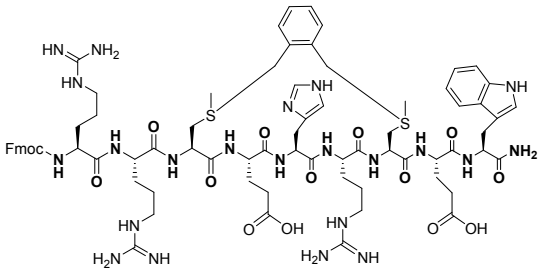

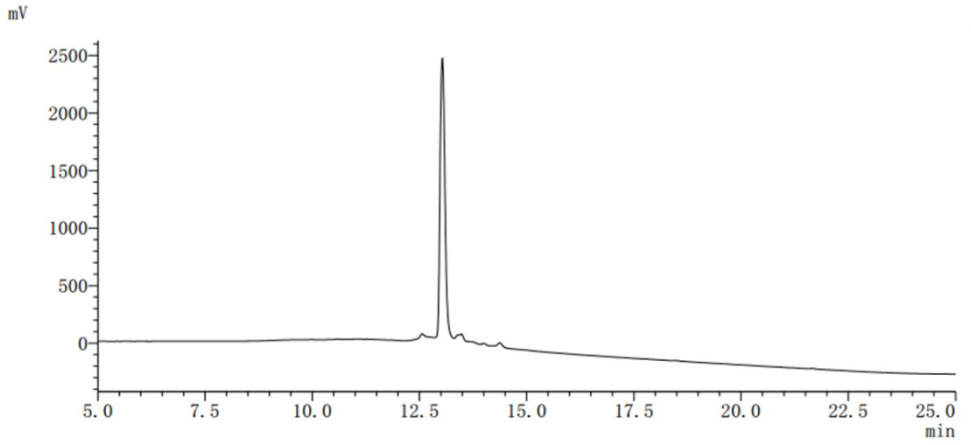

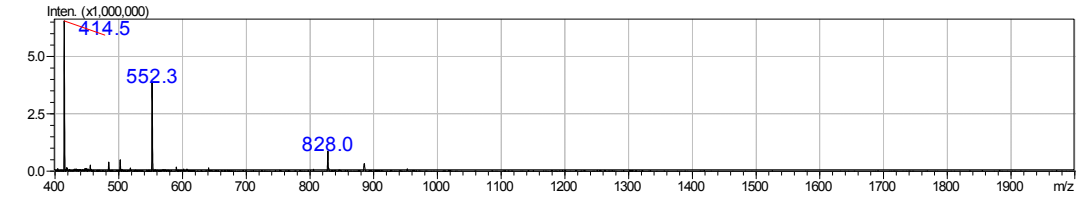

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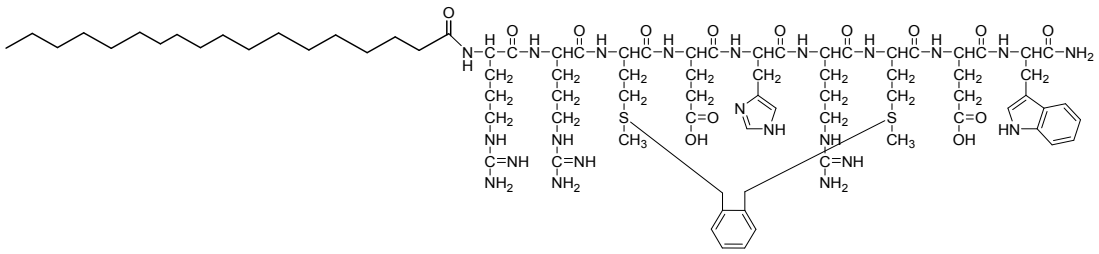

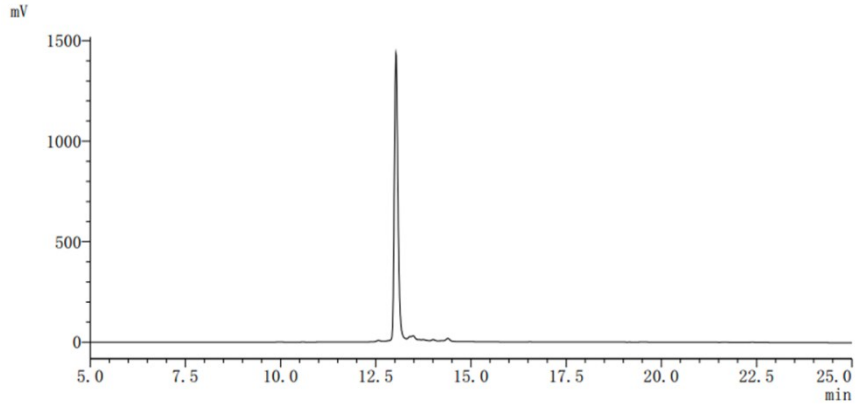

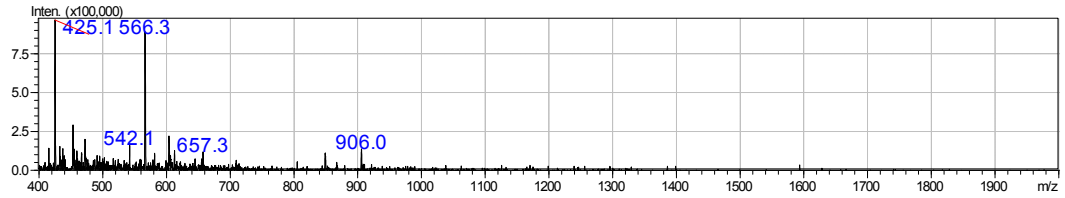

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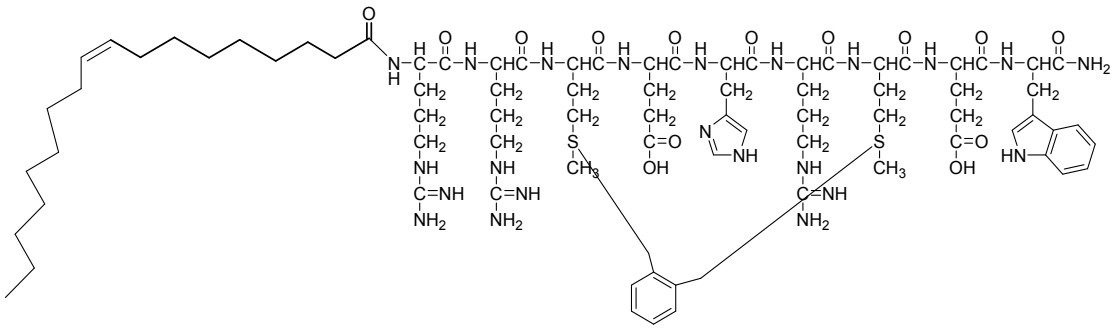

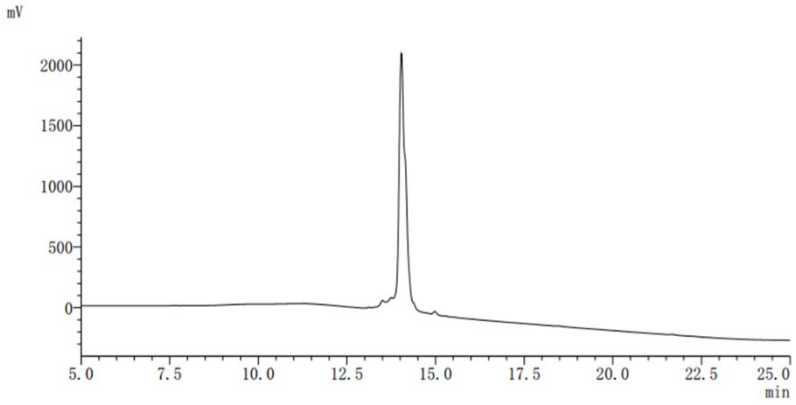

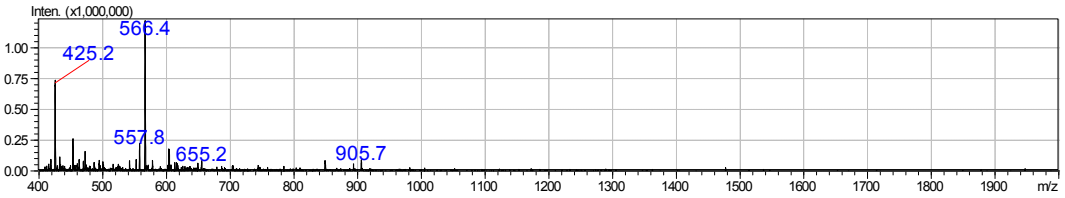

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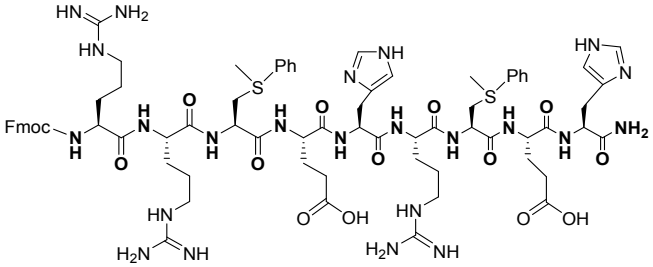

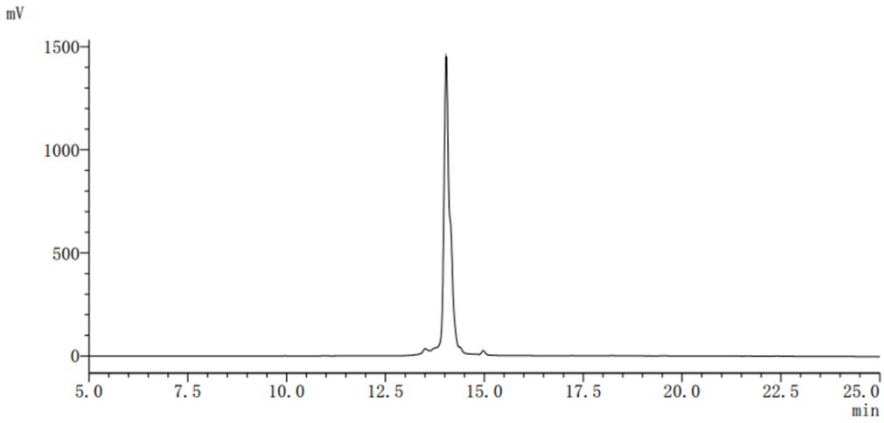

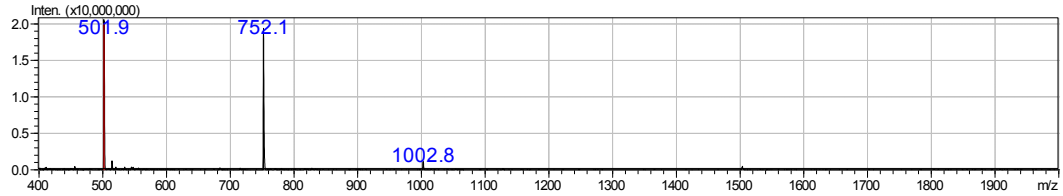

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