

Multifunctional gene delivery vectors containing different liver-targeting fragments for specifically transfecting hepatocellular carcinoma (HCC) cells

Qin Cheng ^{a, b, 1}, Taoran Wang ^{a, 1}, Jing Zhang ^{a, b}, Long Tian ^{a, c}, Chunlan Zeng ^a, Zhao Meng ^{a, *}, Changhao Zhang ^{b, *}, Qingbin Meng ^{a, c, *}

^a State Key laboratory of Toxicology and Medical Countermeasures, Beijing institute of Pharmacology and Toxicology, Beijing, 100850, China

^b Key laboratory of Natural Medicines of the Changbai Mountain, Ministry of education, College of Pharmacy, Yanbian University, Yanji, 133002, China

^c Key Laboratory of Structure-Based Drug Design and Discovery of the Ministry of Education, Shenyang Pharmaceutical University, Shenyang, 110016, China

¹ These authors contributed to the work equally.

* Corresponding author.

Author Email Address:

Zhao Meng: mengzhao900817@163.com

Changhao Zhang: zhangch@ybu.edu.cn

Qingbin Meng: nankaimqb@sina.com

Table S1. Sequences and molecular weights (MWs) of peptides.

Figure S1. RP-HPLC of peptide vectors.

Figure S2. MALDI-TOF-MS or ESI-MS of peptide vectors.

Figure S3. Agarose gel electrophoresis assays of peptide vectors at different N/P ratios.

Figure S4. TEM images of peptide/DNA complexes at the N/P ratio of 10. The scale bar represents 200 nm.

Table S2. The concentrations of peptide/DNA complexes in the cytotoxicity analysis.

Figure S5. Flow cytometric analysis of cellular uptake mechanisms of H-02/DNA (A) and H-09/DNA (B) complexes in HepG2 cells using specific endocytosis inhibitors.

The DNA was labeled with YOYO-1.

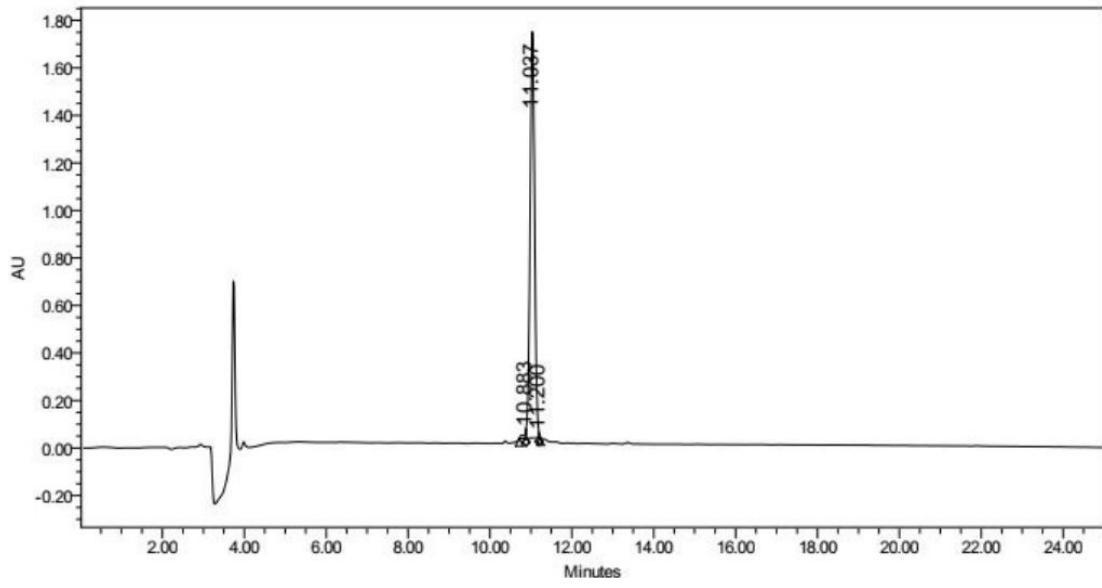
Table S3. The mean intensity of the green fluorescence in HepG2 and LO2 cells measured by ImageJ in CLSM analysis.

Table S4. The CLR of DAPI and YOYO-1 in live-cell imaging experiments.

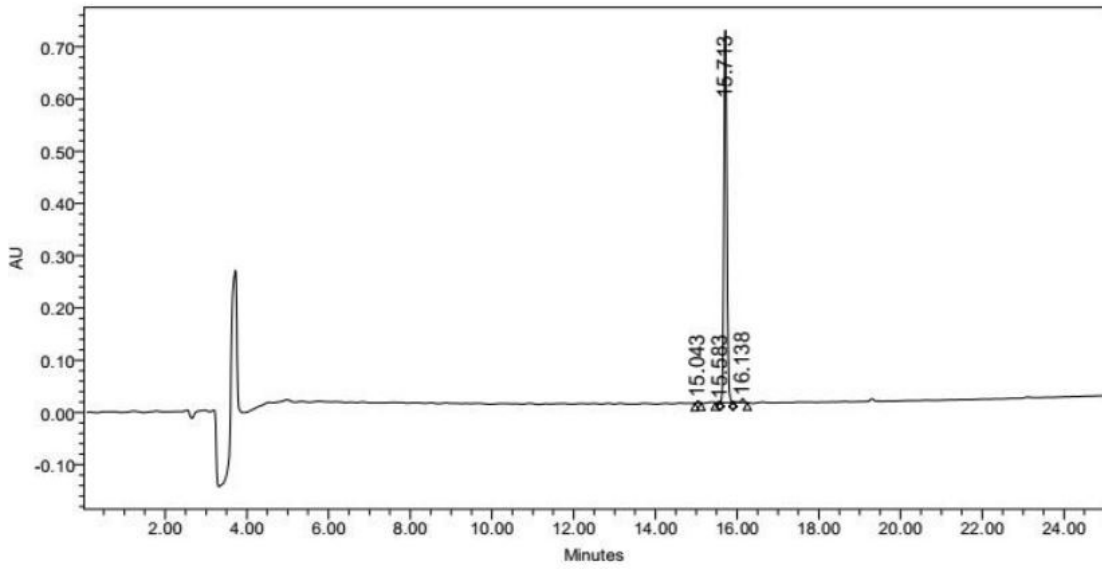
Table S1. Sequences and molecular weights of peptides.

Compounds	Peptide sequences	MWs	
		Calculated	Measured
H-01	K(C ₁₈)-(LLHH) ₃ -R ₉	3359.38	3361.07
H-02	KVGGNY-K(C ₁₈)-(LLHH) ₃ -R ₉	3937.82	3938.32
H-03	KVGGNYNYLYRLF-K(C ₁₈)-(LLHH) ₃ -R ₉	4907.94	4909.86
H-04	NGVEGFN-K(C ₁₈)-(LLHH) ₃ -R ₉	4036.86	4037.77
H-05	HAIYPRH-K(C ₁₈)-(LLHH) ₃ -R ₉	4194.12	4191.07
H-06	AHLHNRS-K(C ₁₈)-(LLHH) ₃ -R ₉	4135.00	4132.08
H-07	DYEMHLWWGTEL-K(C ₁₈)-(LLHH) ₃ -R ₉	4880.84	4877.56
H-08	THVSPNQGLPS-K(C ₁₈)-(LLHH) ₃ -R ₉	4494.38	4491.35
H-09	KSLSRHDHIIHHH-K(C ₁₈)-(LLHH) ₃ -R ₉	4804.75	4803.59
H-10	SFSIIHTPILPL-K(C ₁₈)-(LLHH) ₃ -R ₉	4638.72	4635.55
H-11	GNY-K(C ₁₈)-(LLHH) ₃ -R ₉	3653.46	3652.47
H-12	GVKYNG-K(C ₁₈)-(LLHH) ₃ -R ₉	3937.82	3934.97
H-13	DHIIHHH-K(C ₁₈)-(LLHH) ₃ -R ₉	4095.95	4095.23
H-14	HKHDSHLISHRH-K(C ₁₈)-(LLHH) ₃ -R ₉	4804.75	4801.88
H-15	KVGGNY	635.72	637.05
H-16	KSLSRHDHIIHHH	1502.65	1502.47
H-17	K(C ₁₈)-C(Cy5.5)-(LLHH) ₃ -R ₉	4127.11	4131.12
H-18	KSLSRHDHIIHHH-K(C ₁₈)-C(Cy5.5)-(LLHH) ₃ -R ₉	5612.74	5617.75

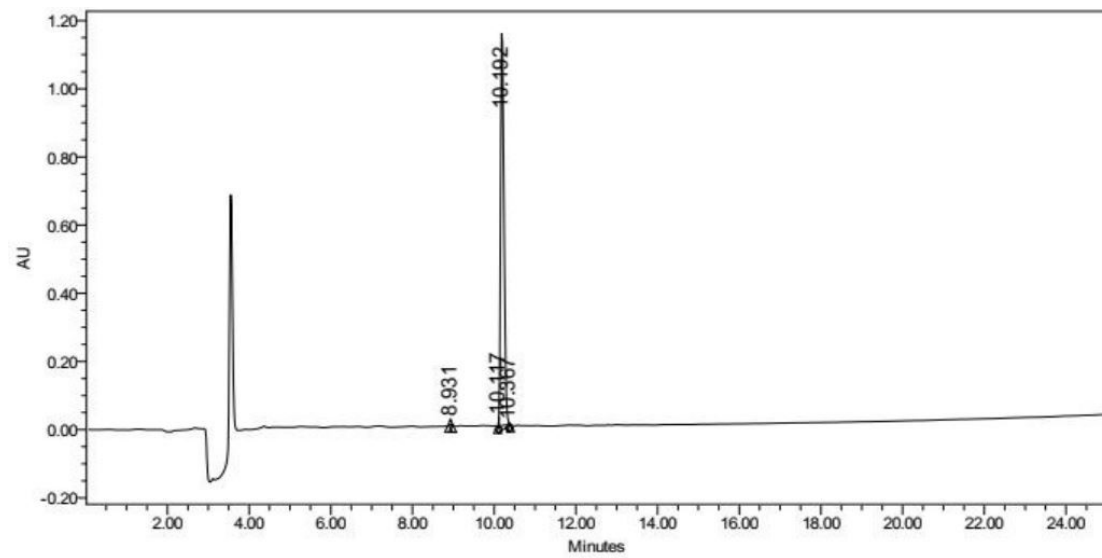
H-01



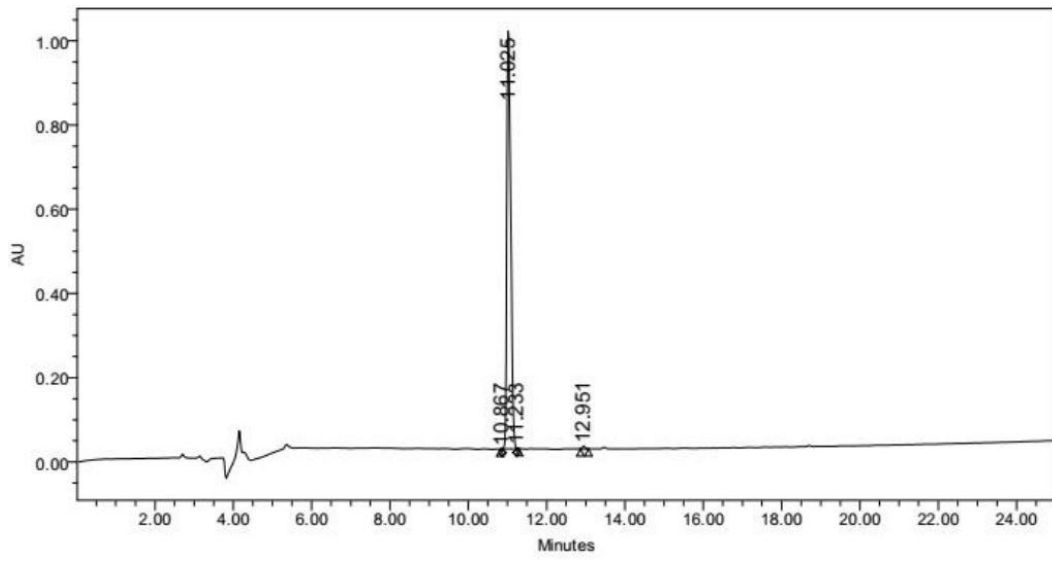
H-02



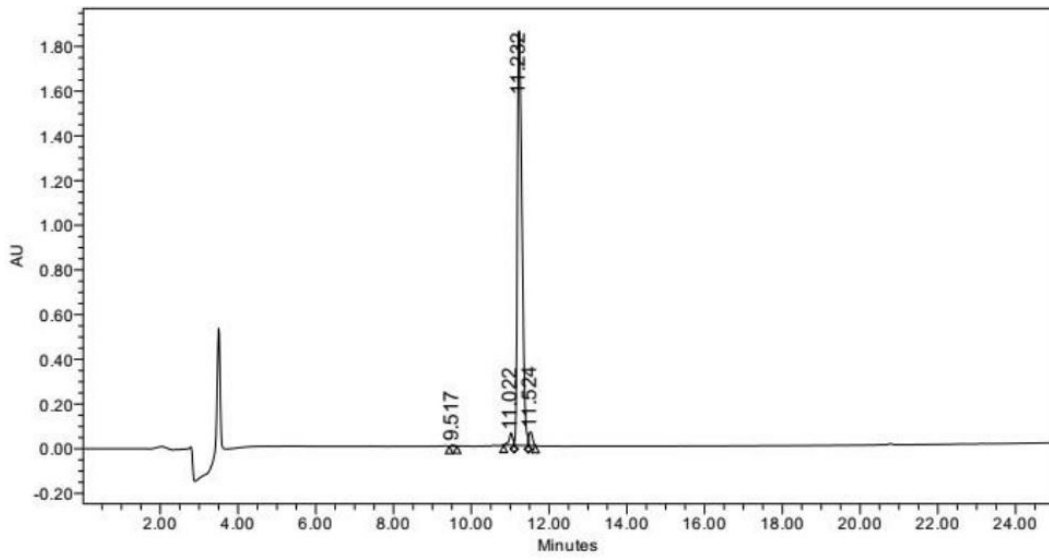
H-03



H-04

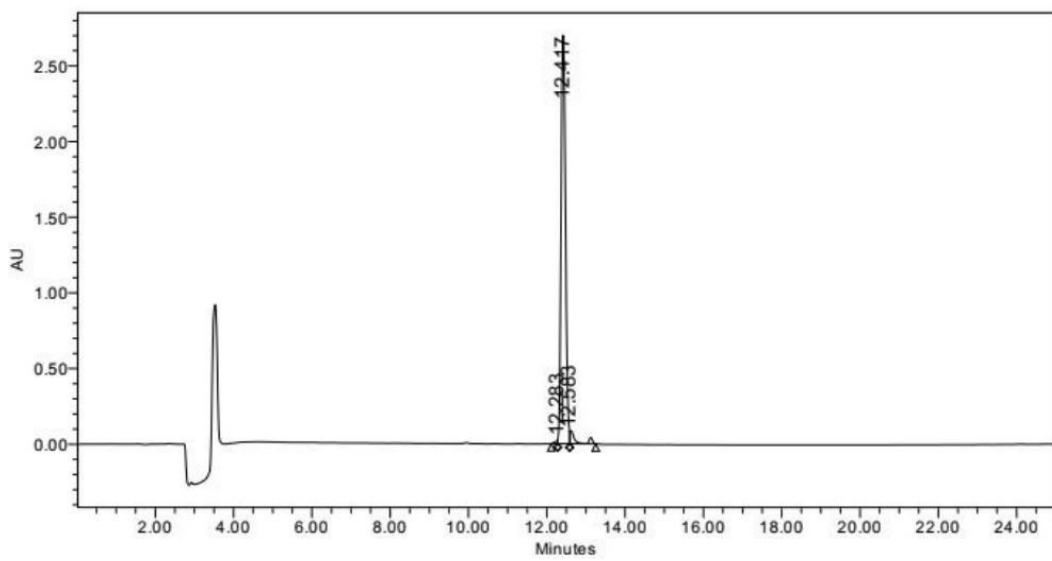


H-

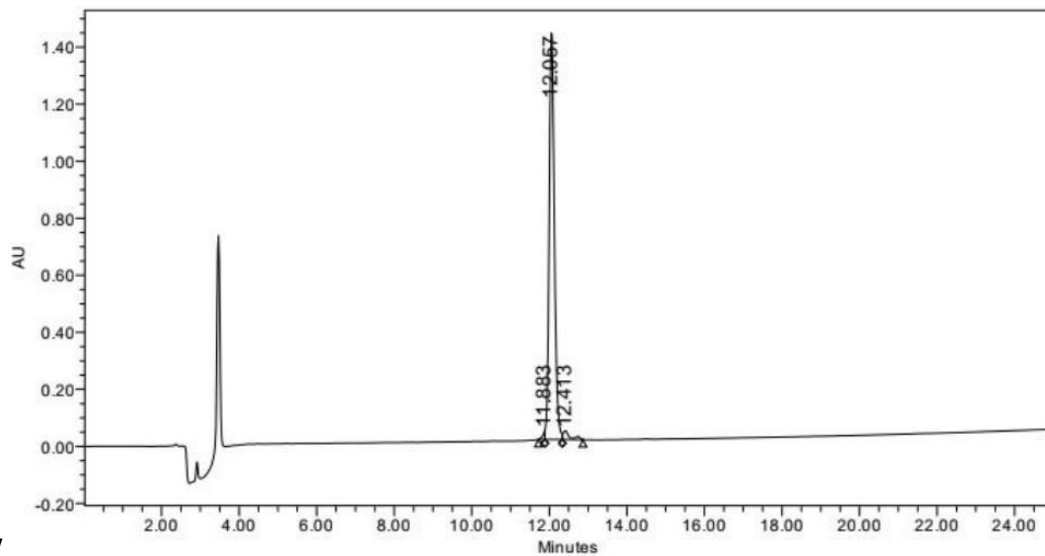


05

H-06

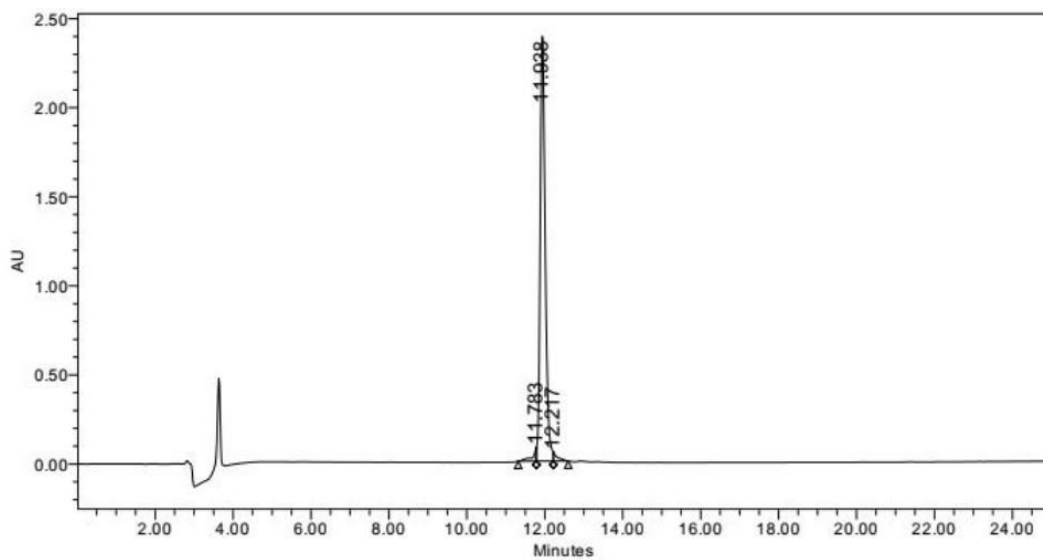


H-

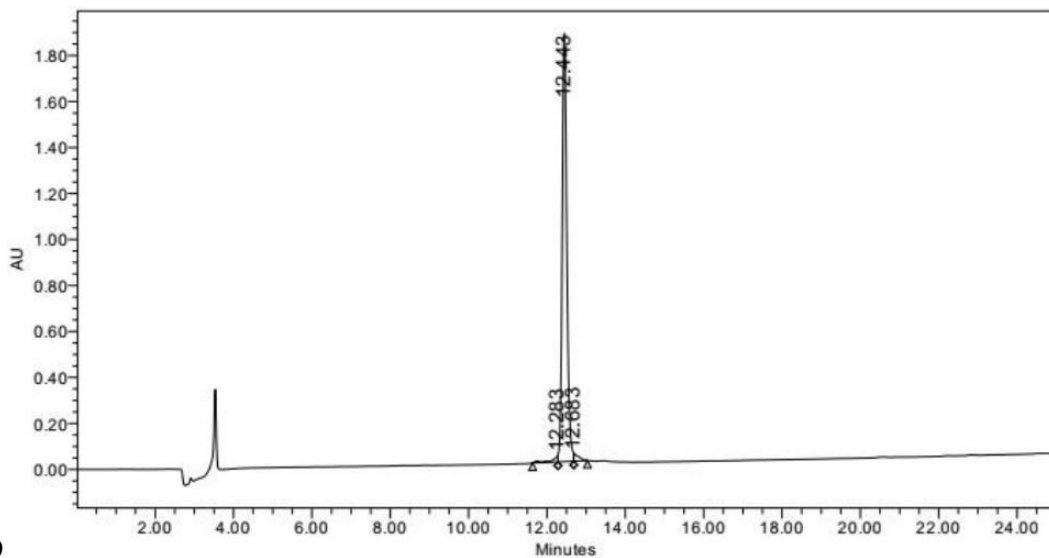


07

H-08

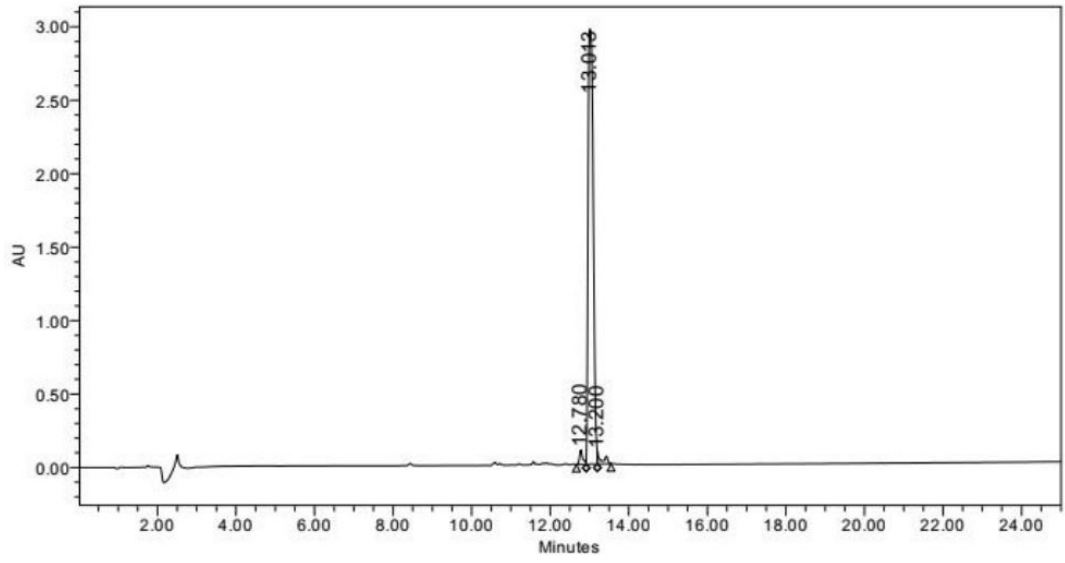


H-

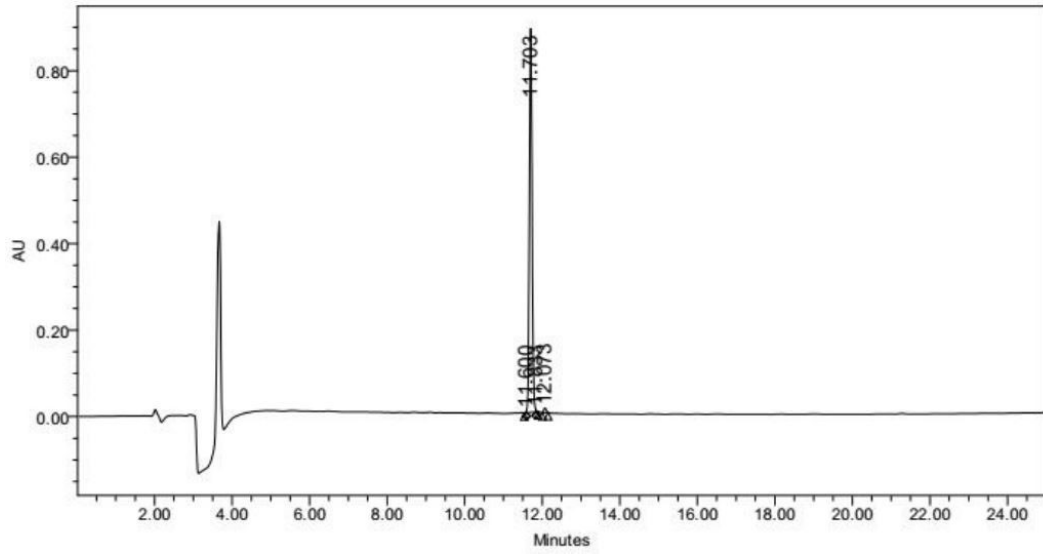


09

H-10

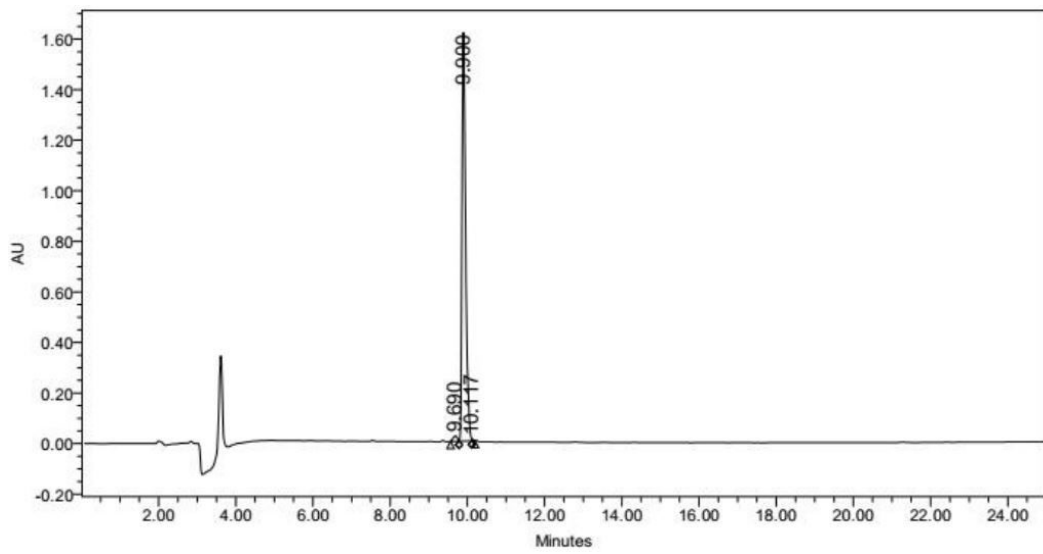


H-

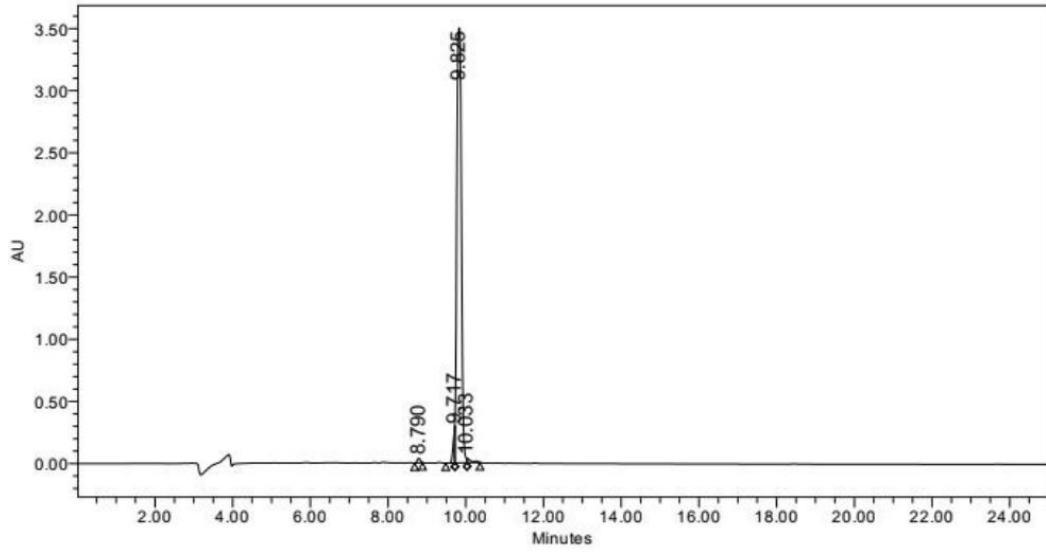


11

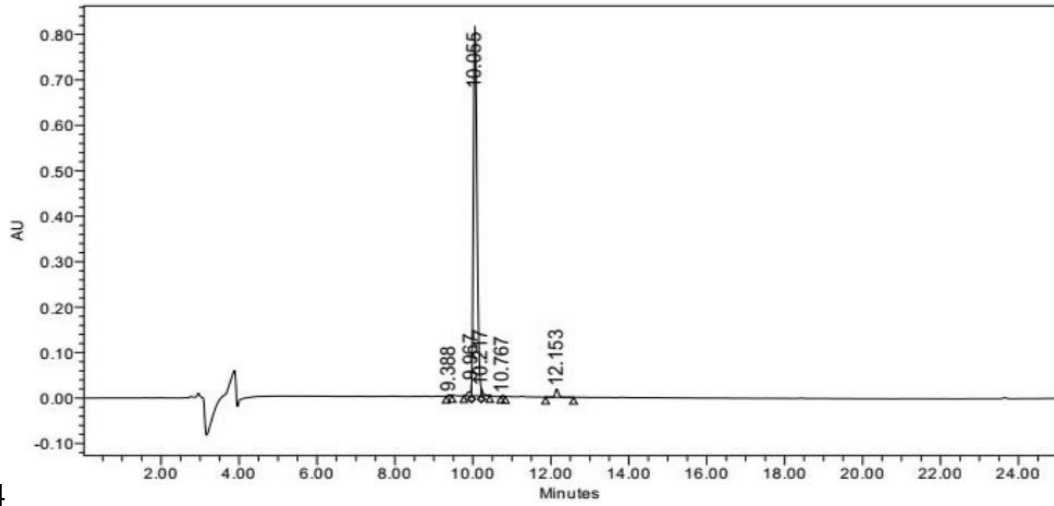
H-12



H-13

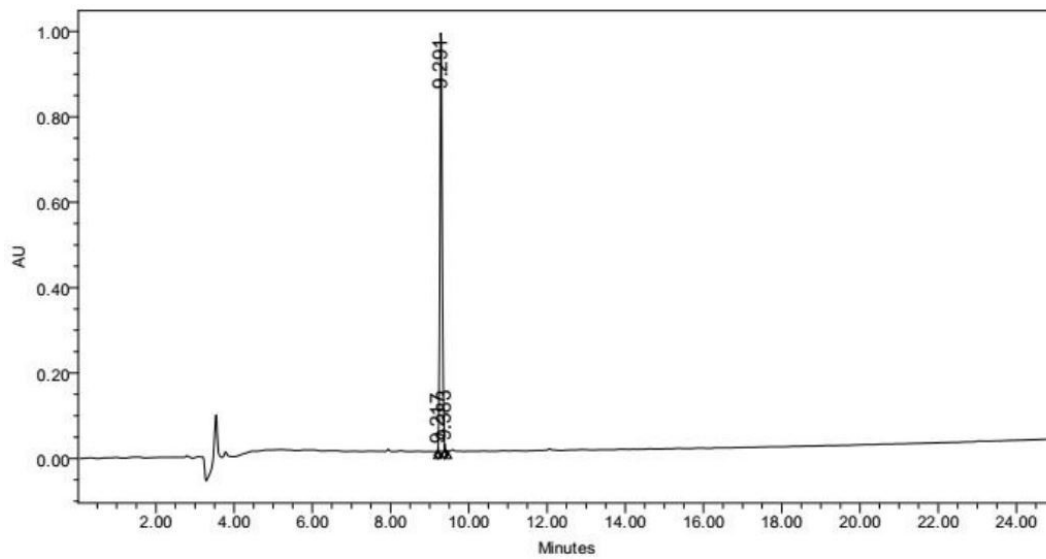


H-



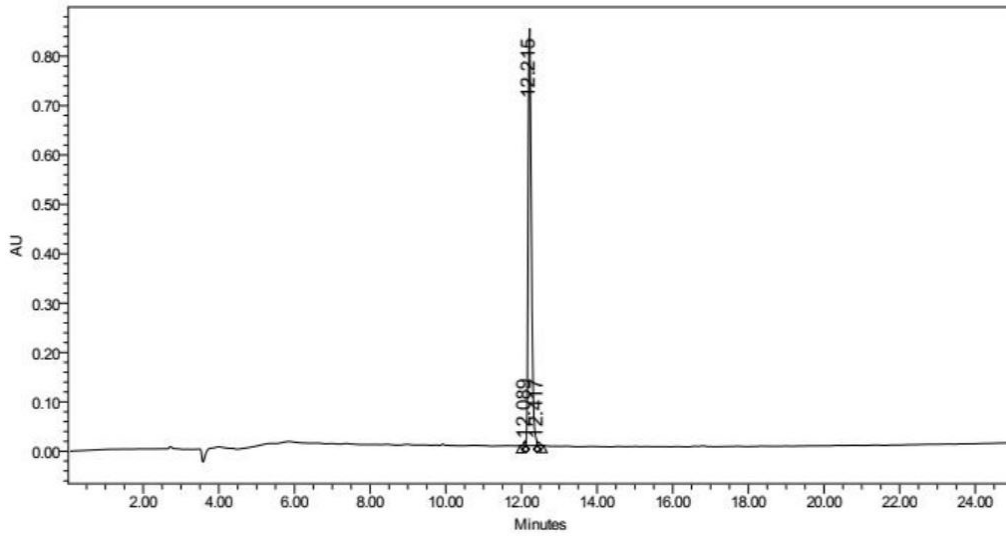
14

H-15

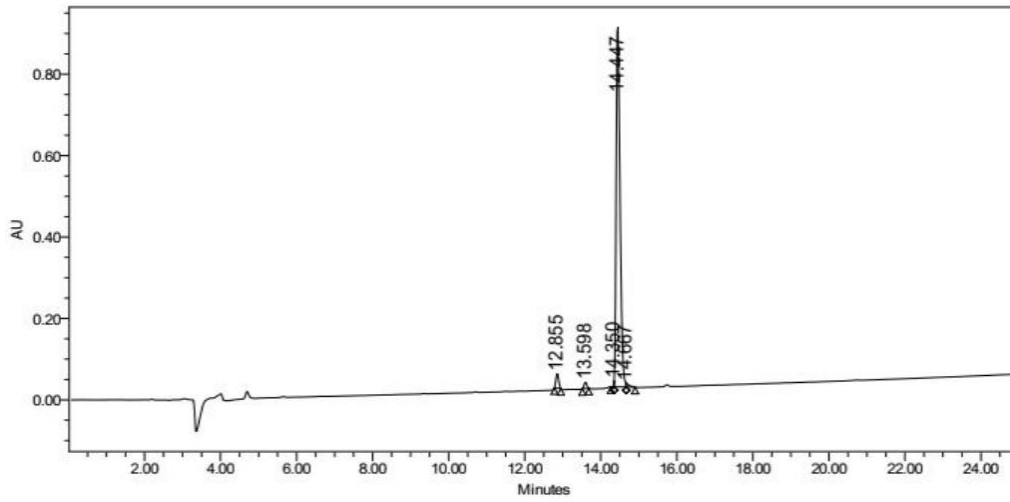


H-

16



H-17



H-18

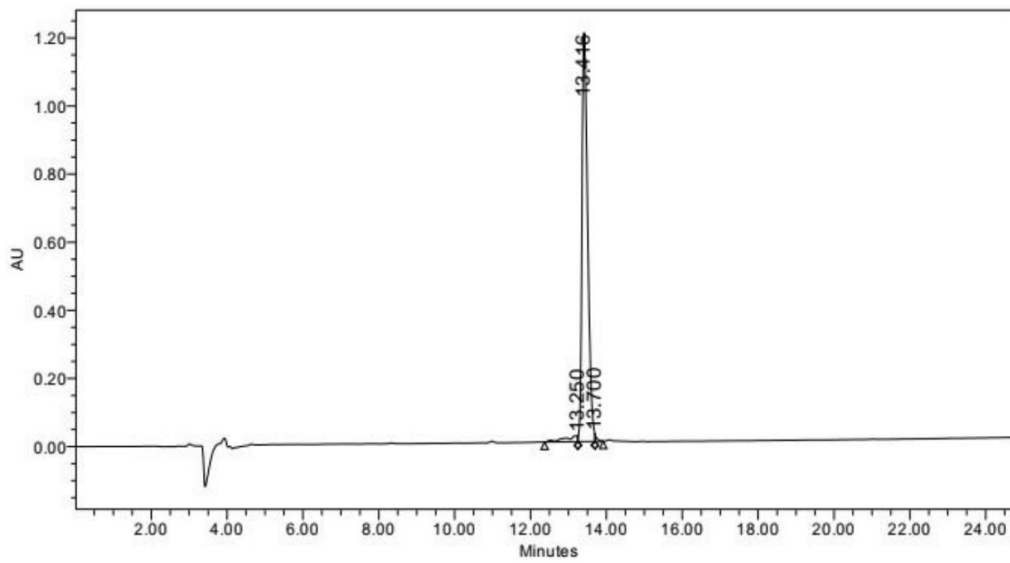
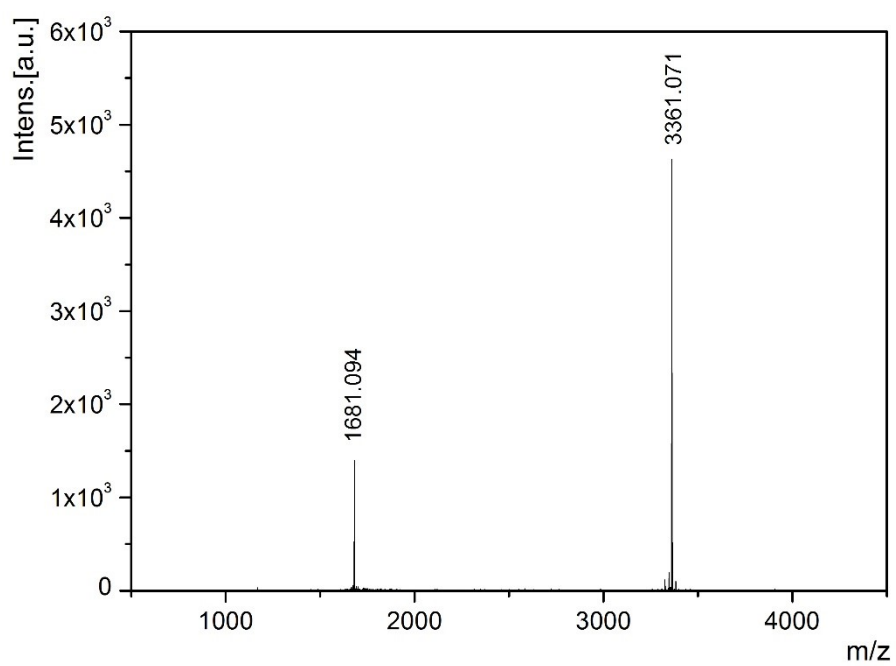
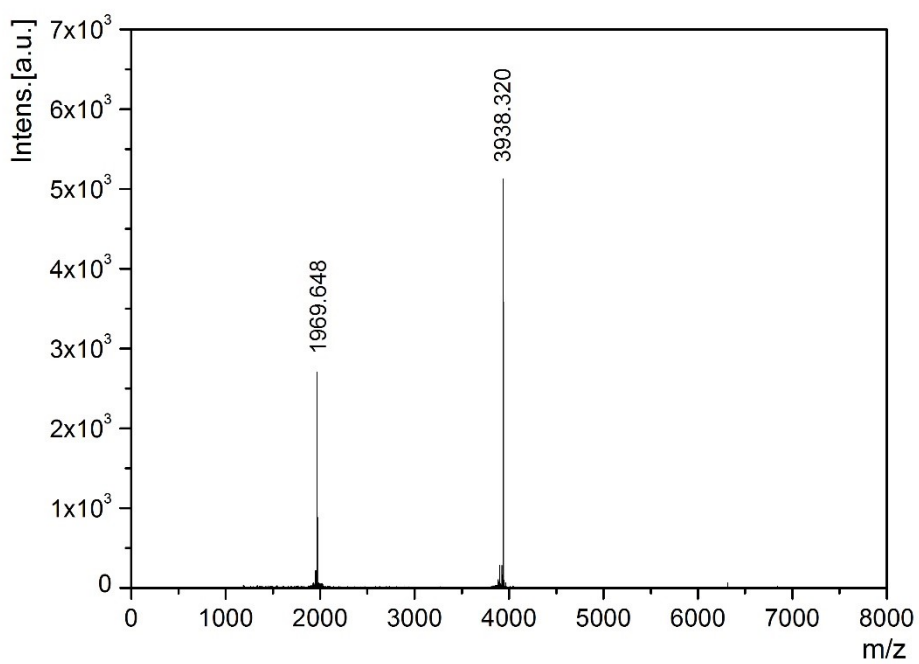


Figure S1. RP-HPLC of peptide vectors.

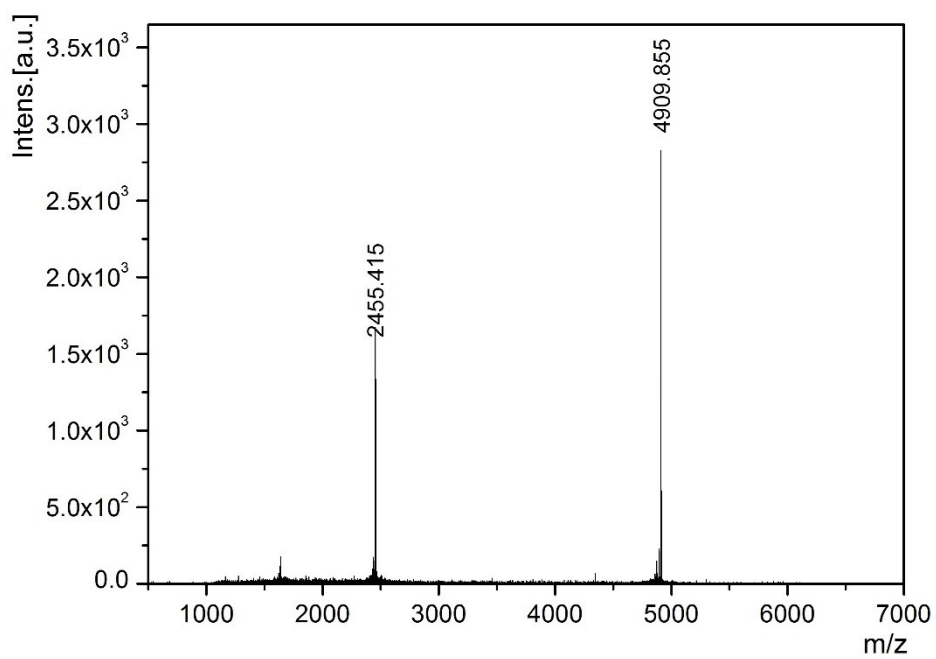
H-01



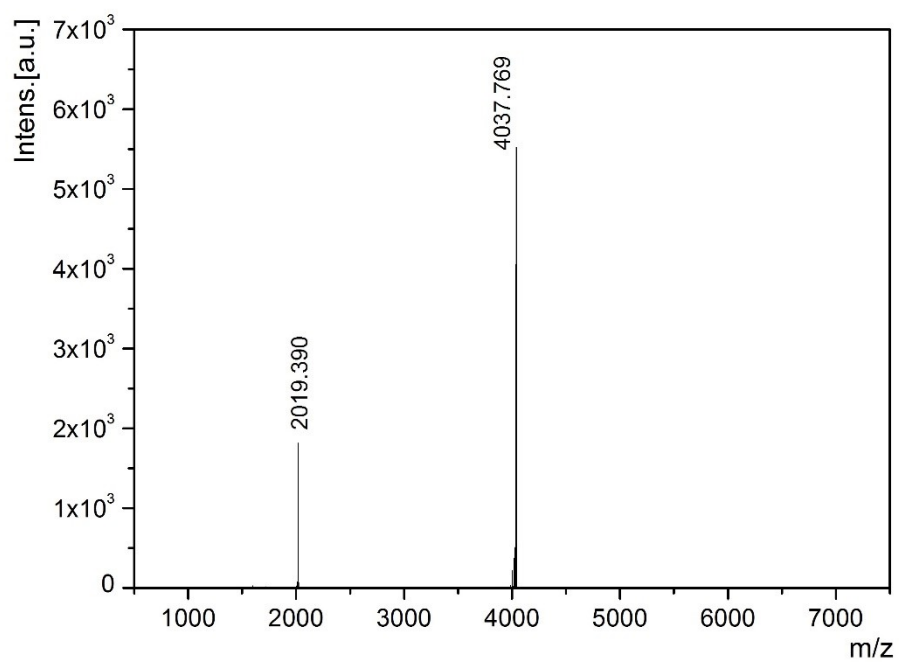
H-02



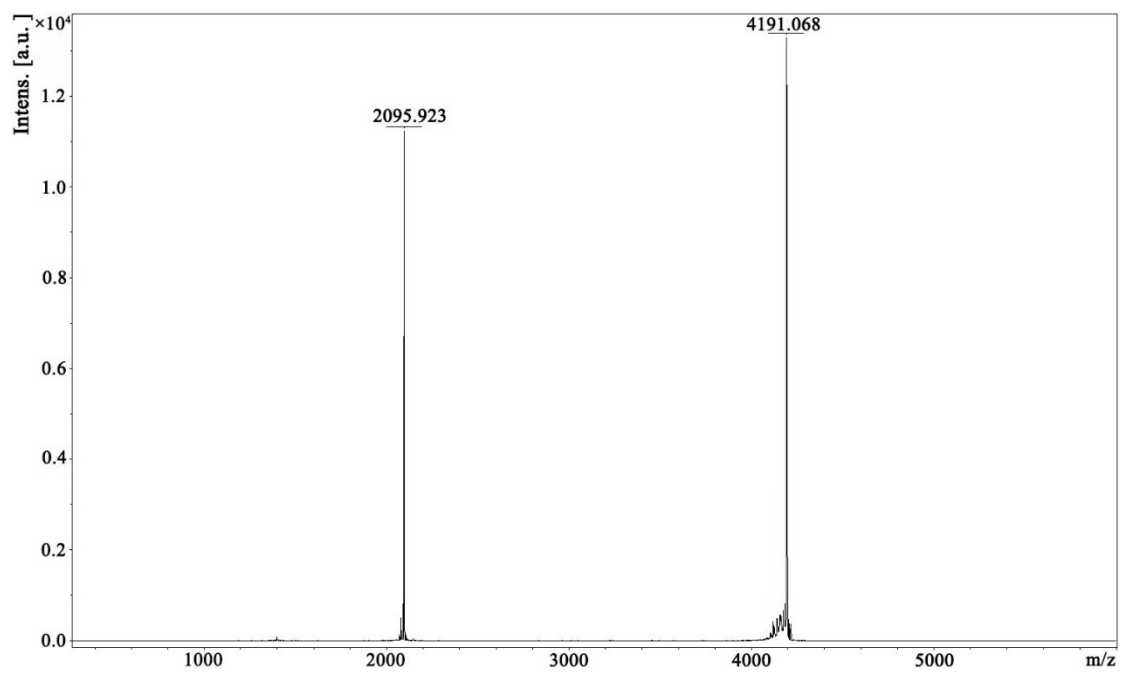
H-03



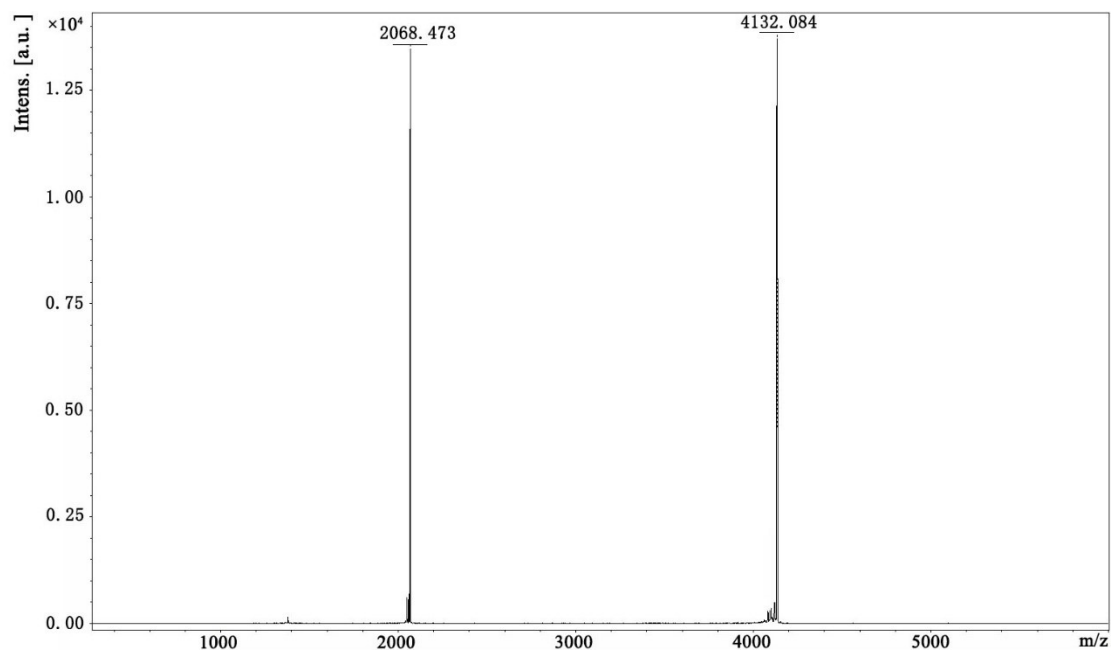
H-04



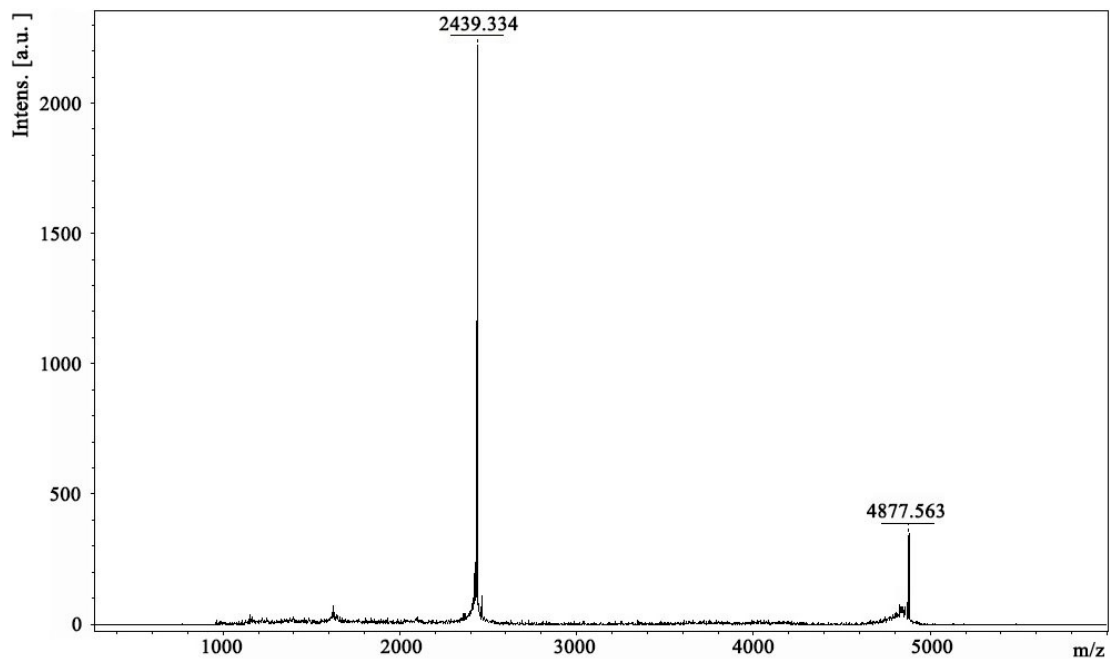
H-05



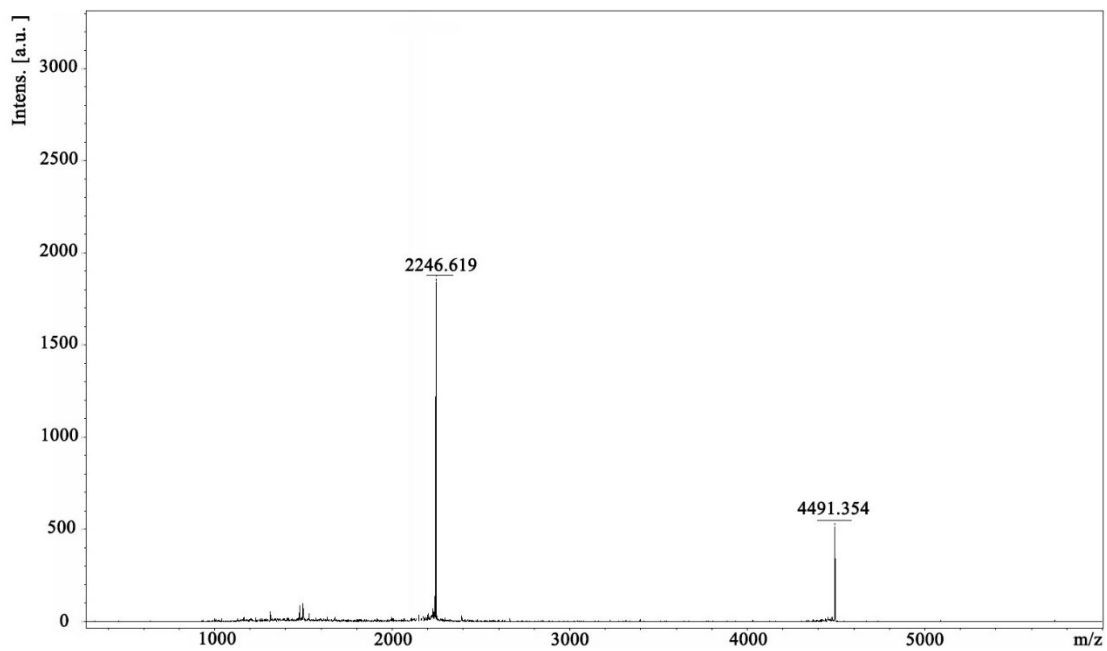
H-06



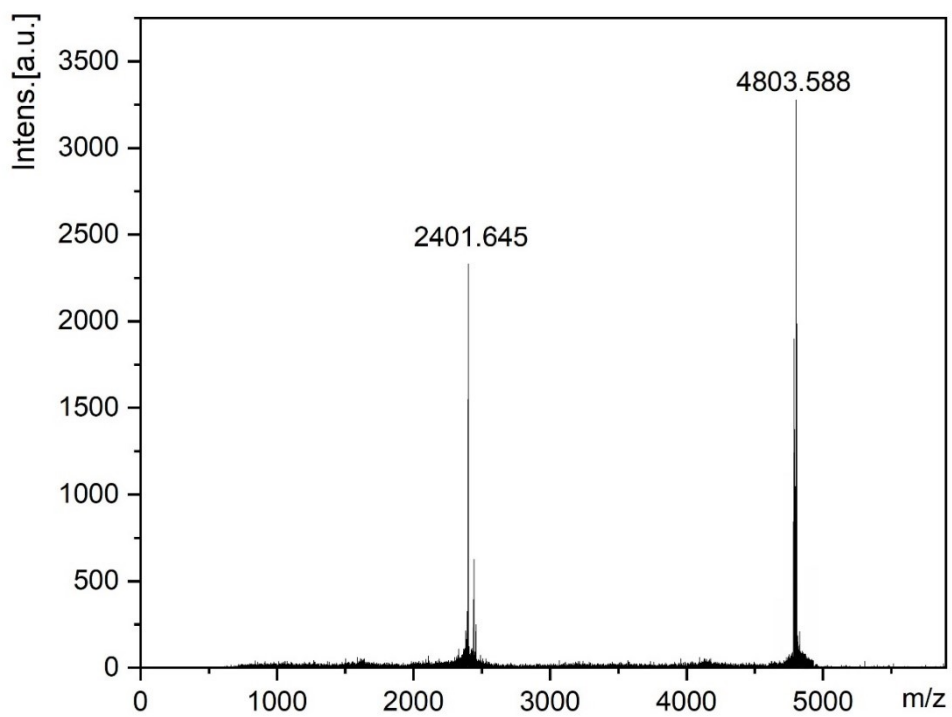
H-07



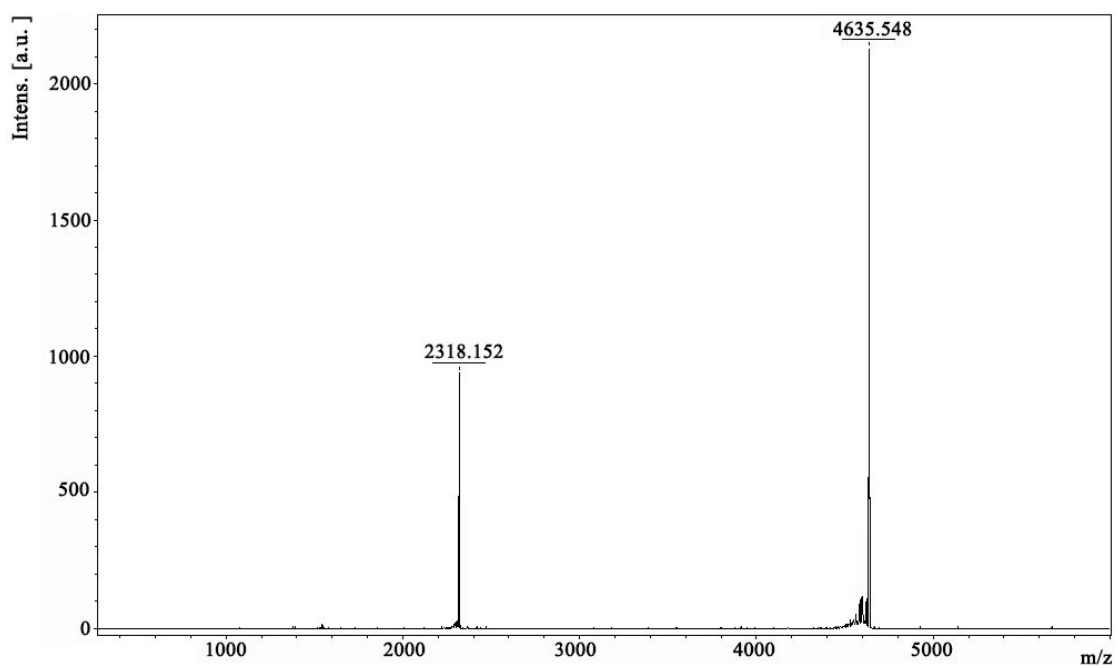
H-08



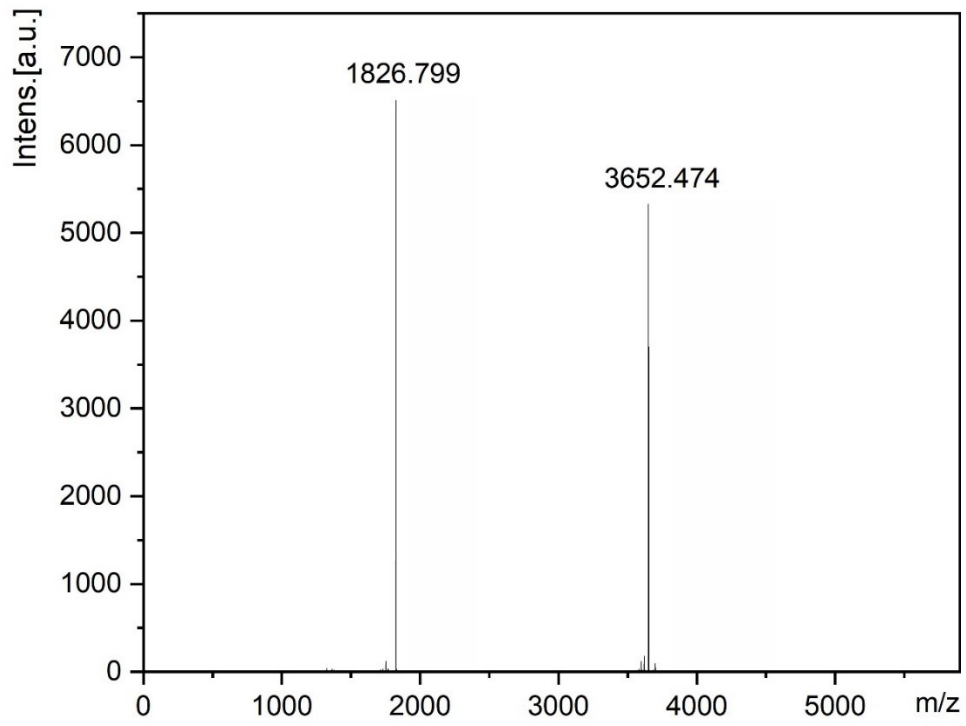
H-09



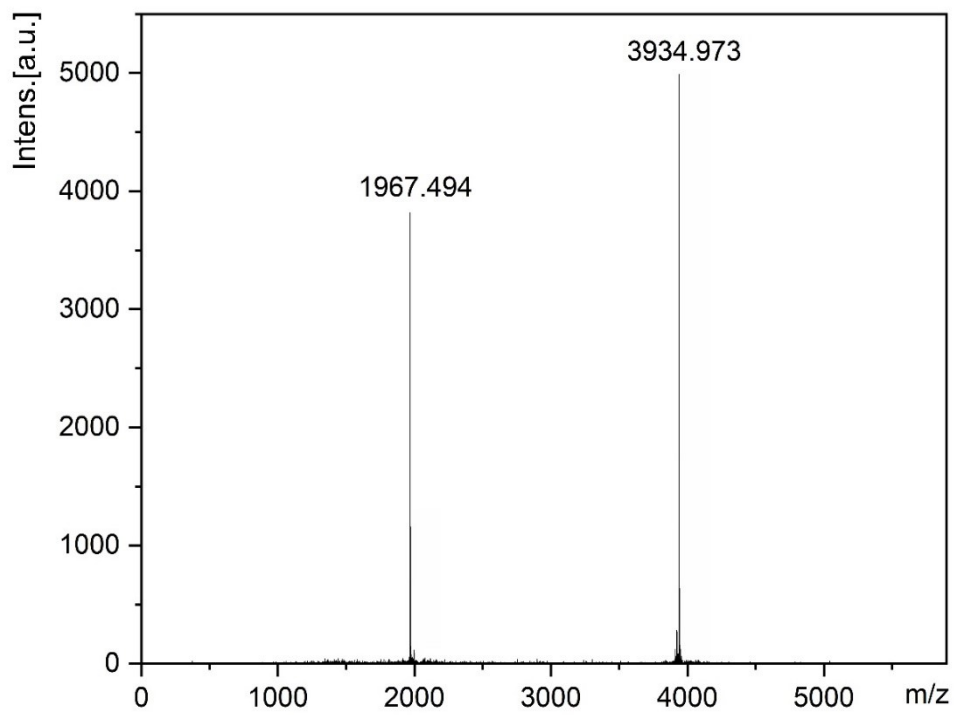
H-10



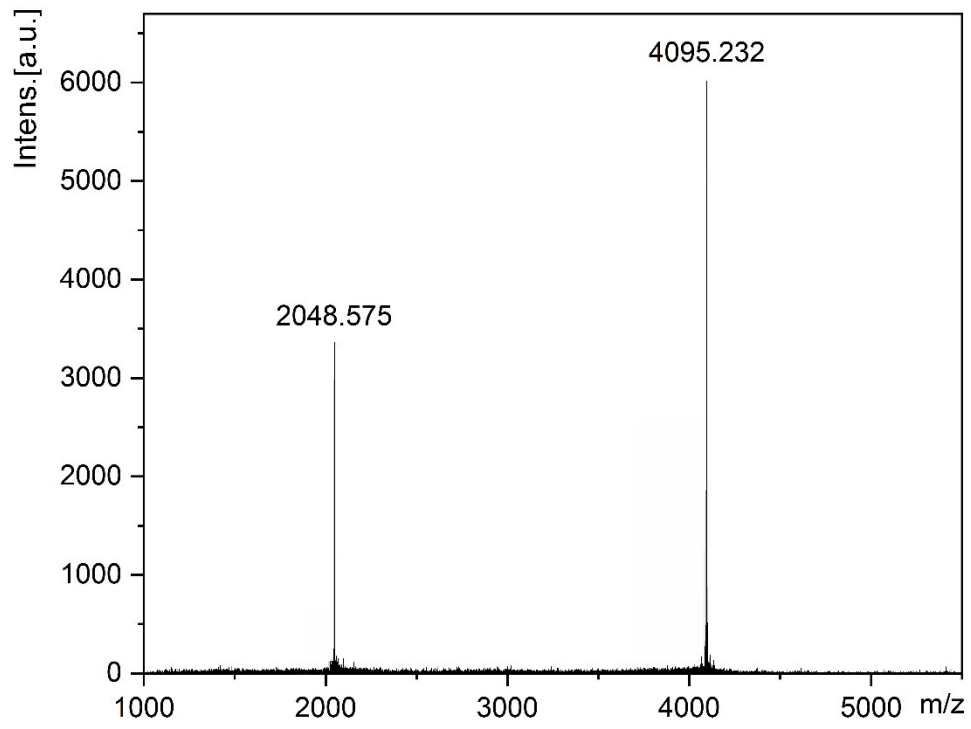
H-11



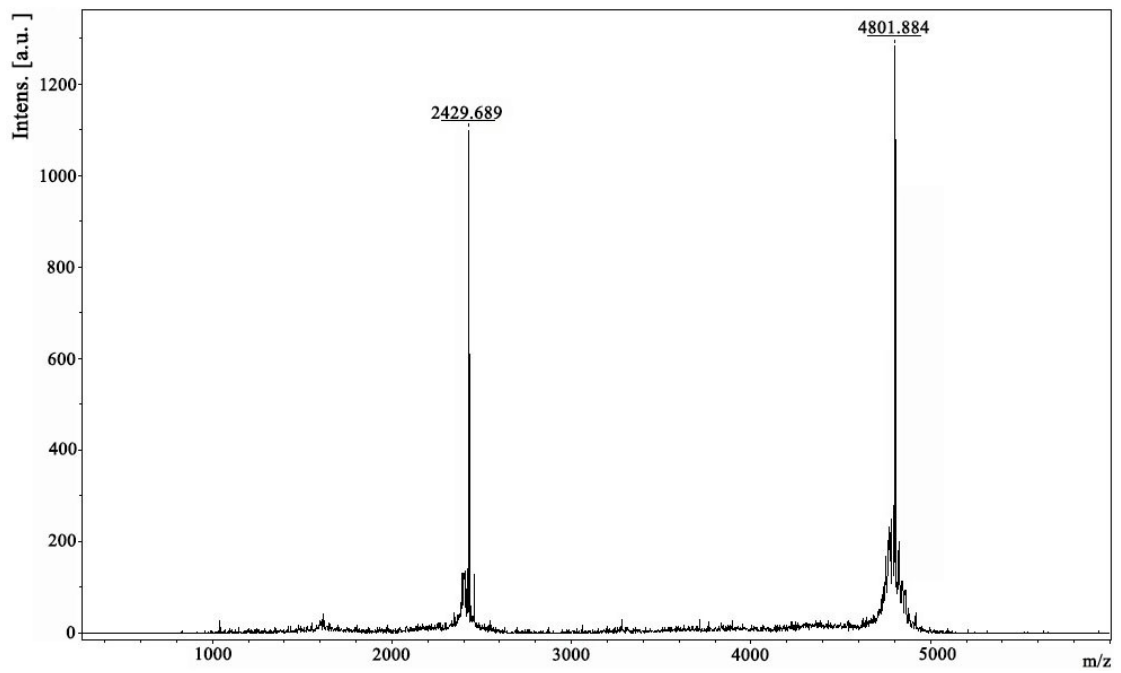
H-12



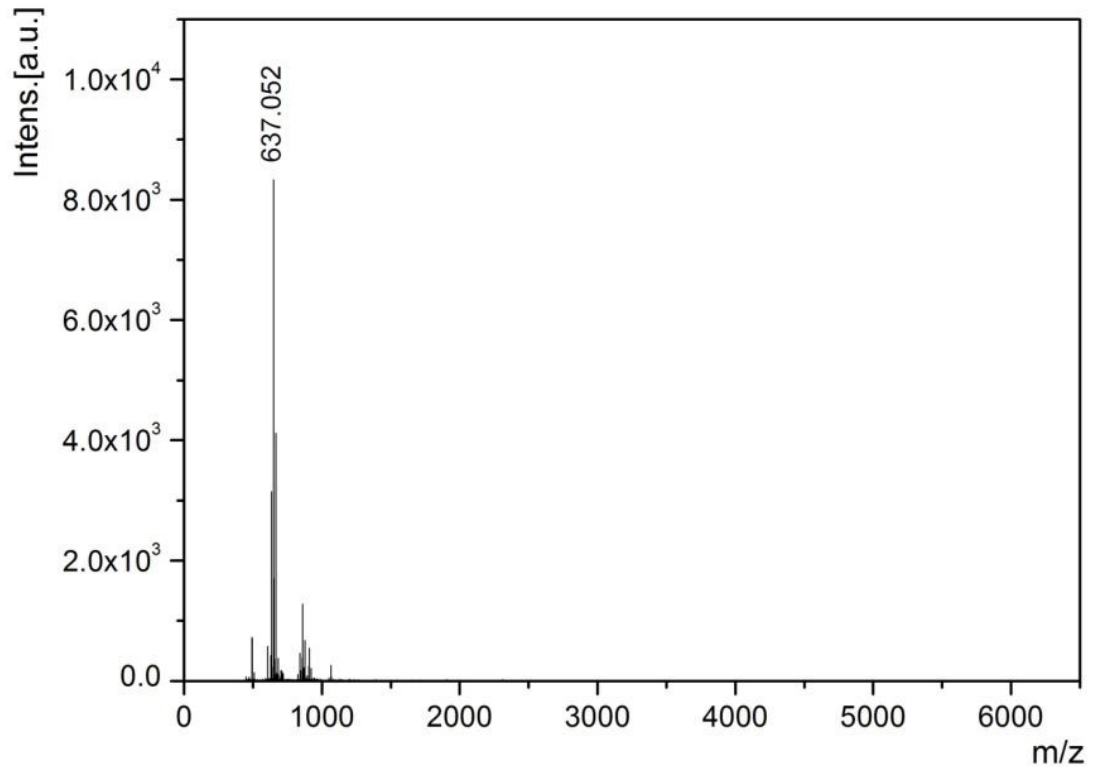
H-13



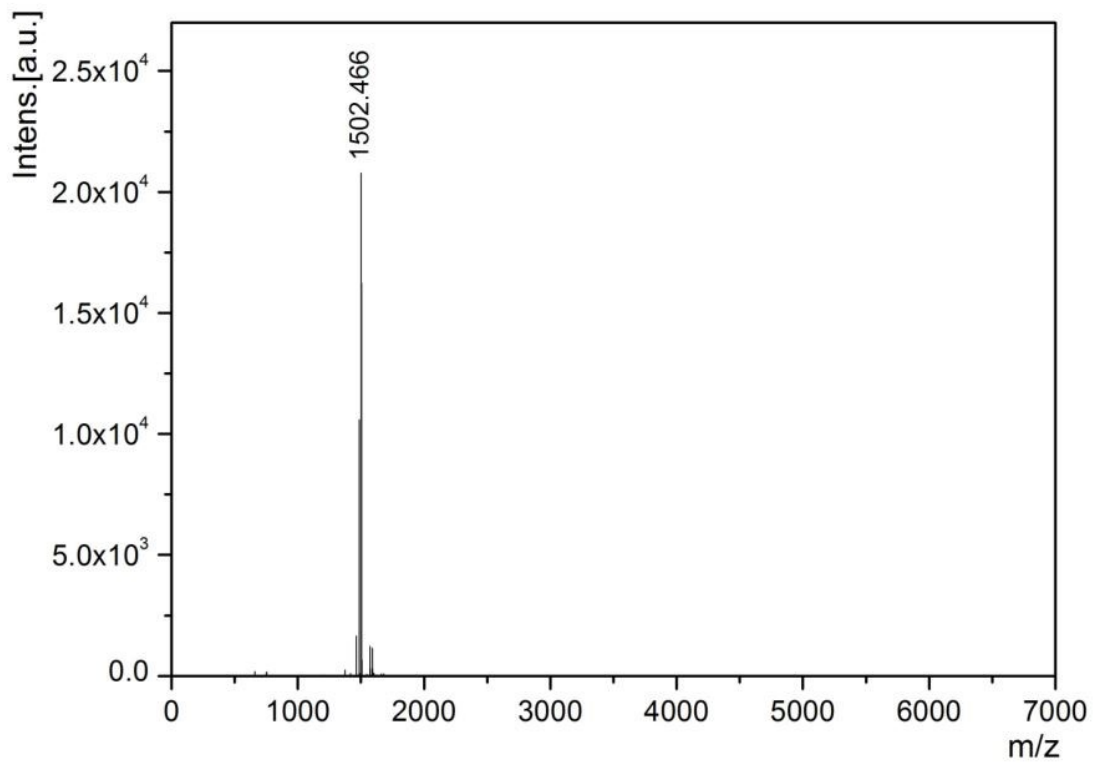
H-14



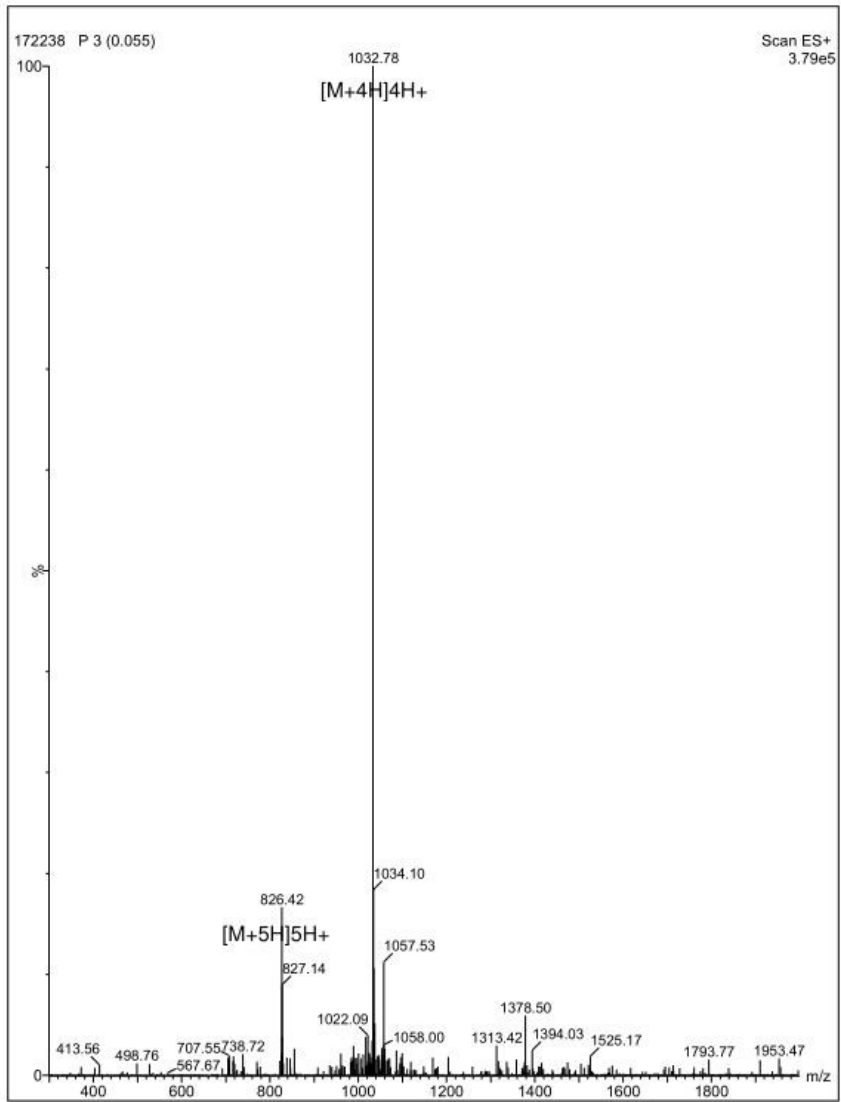
H-15



H-16



H-17



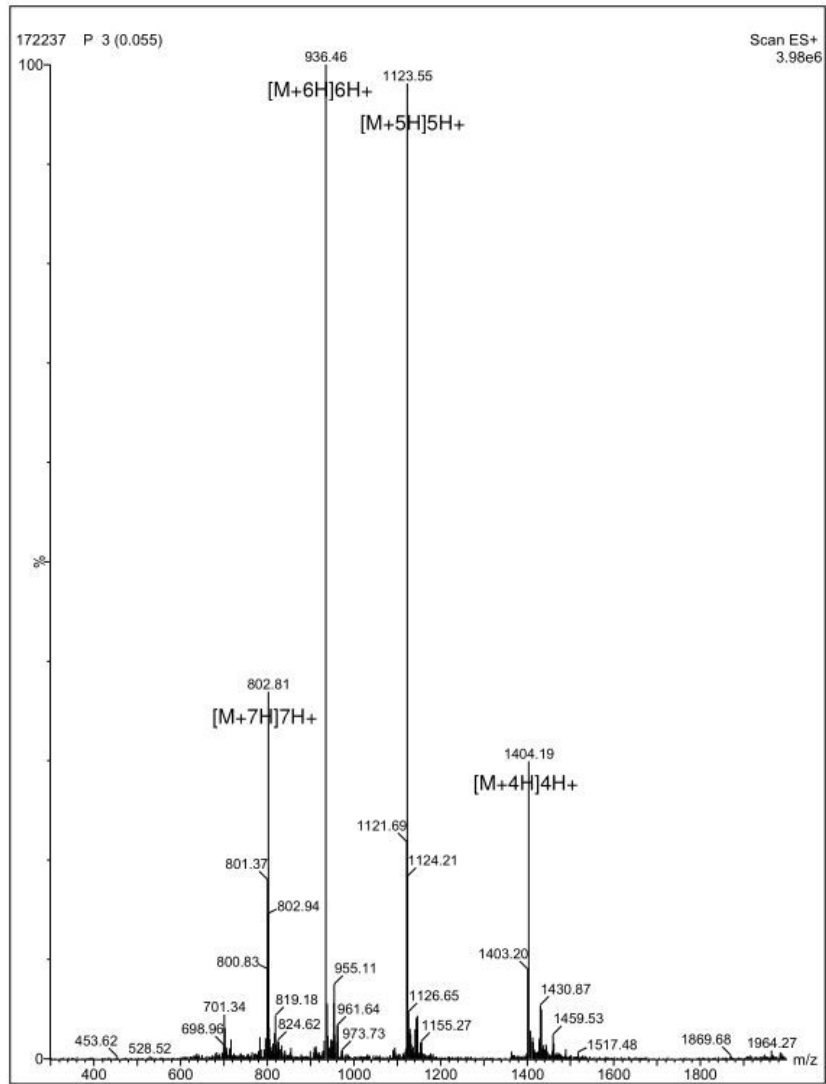


Figure S2. MALDI-TOF-MS or ESI-MS of peptide vectors.

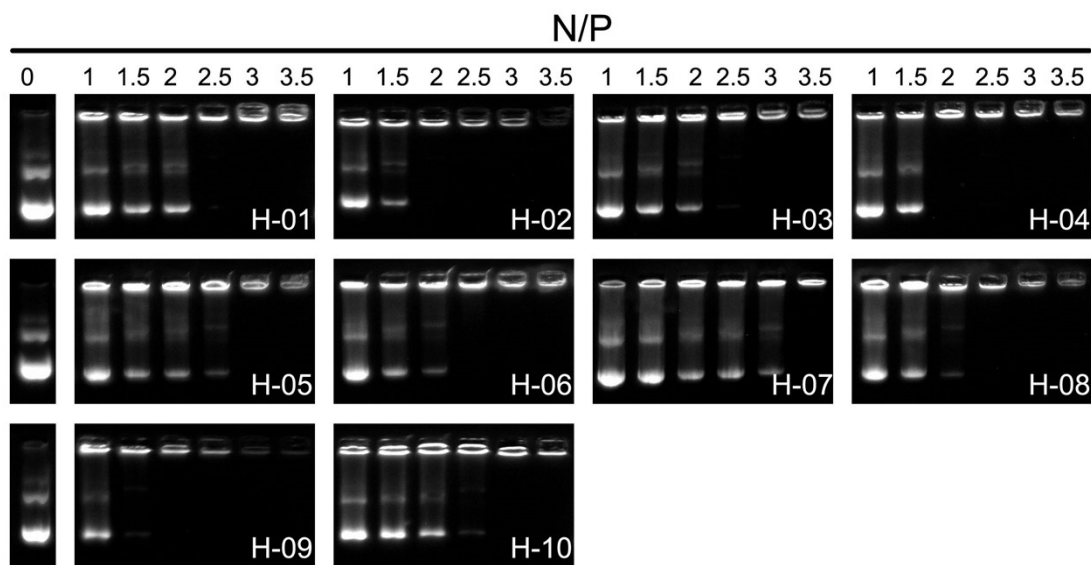


Figure S3. Agarose gel electrophoresis assays of peptide vectors at different N/P ratios.

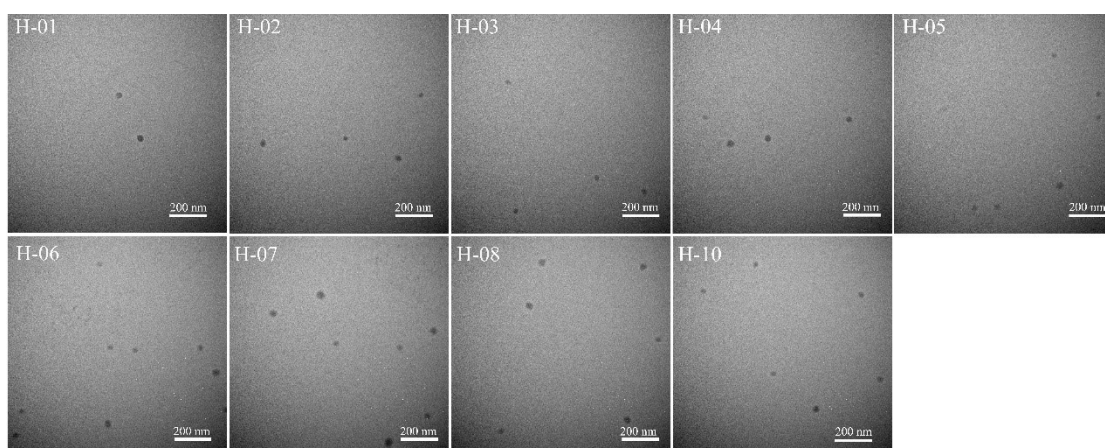


Figure S4. TEM images of peptide/DNA complexes at the N/P ratio of 10. The scale bar represents 200 nm.

Table S2. The concentrations of peptide/DNA complexes in the cytotoxicity analysis

Compounds	Concentrations (μM)				
	N/P				
	4	6	8	10	12
H-01	1.37	2.05	2.74	3.42	4.11
H-02	1.23	1.85	2.46	3.08	3.70
H-03	1.12	1.68	2.24	2.80	3.36
H-04	1.54	2.31	3.08	3.85	4.62
H-05	1.23	1.85	2.46	3.08	3.85
H-06	1.23	1.85	2.46	3.08	3.85
H-07	2.05	3.08	4.11	5.13	6.16
H-08	1.37	2.05	2.74	3.42	4.11
H-09	1.23	1.85	2.46	3.08	3.70
H-10	1.37	2.05	2.74	3.42	4.11

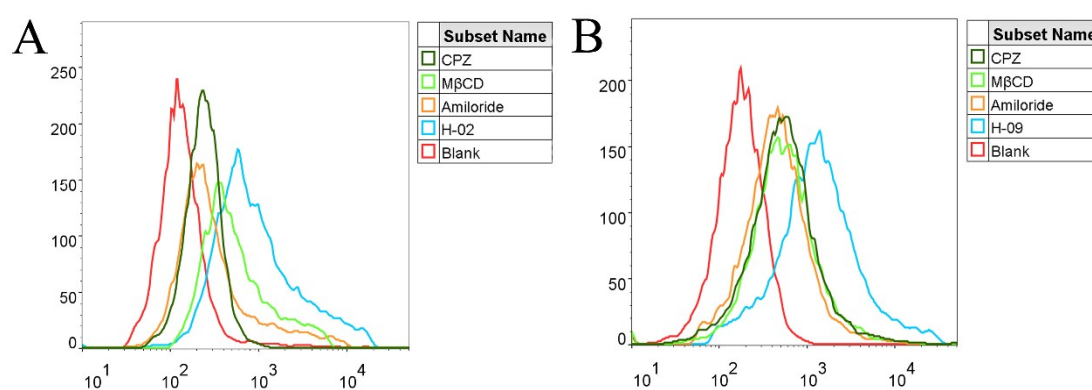


Figure S5. Flow cytometric analysis of cellular uptake mechanisms of H-02/DNA (A) and H-09/DNA (B) complexes in HepG2 cells using specific endocytosis inhibitors. The DNA was labeled with YOYO-1.

Table S3. The mean intensity of the green fluorescence in HepG2 and LO2 cells measured by ImageJ in CLSM analysis

Compounds	Mean intensity	
	HepG2	LO2
H-01	90.41	117.31
H-02	198.51	139.97
H-03	53.21	57.78
H-04	189.18	158.10
H-05	132.09	83.77
H-06	86.88	88.12
H-07	198.64	85.06
H-08	46.99	83.32
H-09	203.89	122.41
H-10	113.66	61.18
Lipo 2000	53.76	90.87

Table S4. The CLR of DAPI and YOYO-1 in live-cell imaging experiments

Time (min)	CLR (%)			
	H-01	H-02	H-09	Lipo 2000
5	7.33	10.27	16.64	-
15	18.44	20.71	20.72	10.40
60	21.99	47.22	29.94	26.27
120	22.57	49.11	60.56	48.15
240	-	-	-	83.64