Supporting information

Ultrafast protein digestion using immobilized enzyme reactor following high-resolution mass spectrometry analysis for rapid identification of abrin toxin

Meng Chen, Baoqiang Li, Wenlu Wei, Zhongyao Zhang, Lin Zhang, Cuiping Li*, Qibin Huang*

State Key Laboratory of NBC Protection for Civilian, Beijing 102205, China

* Corresponding Authors:

Cuiping Li

State Key Laboratory of NBC Protection for Civilia

Beijing 102205, China

E-mail: cuipingli86@126.com

Qibin Huang

State Key Laboratory of NBC Protection for Civilia

Beijing 102205, China

E-mail: jky301_hxzc@163.com

Tables of Contents

Additional Experimental Details：

1. Material and reagents
2. Instruments
3. Activity assays of free trypsin and Fe_3O_4@CTS-GA-Try
4. Michaelis-Menten constants of free and immobilized trypsin
5. UHPLC-HRMS anlyisis

Supplementary Tables:

Table S1: Annotated peptides found under the optimal digestion conditions of BSA.

Table S2: Abrin-a (Uniport P11140) measured *m/z* values of trypsin digest peptides found for the digestion of abrin. Mass tolerance was set to ±10 ppm.

Table S3: Abrin-b (Uniport Q06077) measured m/z values of trypsin digest peptides found for the digestion of abrin. Mass tolerance was set to ±10 ppm.

Table S4: Abrin-c (Uniport P28590) measured m/z values of trypsin digest peptides found for the digestion of abrin. Mass tolerance was set to ±10 ppm.

Table S5: Abrin-d (Uniport Q06076) measured m/z values of trypsin digest peptides found for the digestion of abrin. Mass tolerance was set to ±10 ppm.

Table S6: Agglutinin-1 (Uniport Q9M6E9) measured m/z values of trypsin digest peptides found for the digestion of abrin. Mass tolerance was set to ±10 ppm.

Table S7: Comparison between the method reported in this work and previously reported methods for abrin identification.

Supplementary Figures:

Fig. S1: Reaction equation of trypsin-catalyzed hydrolysis of BApNA (A) and ultraviolet spectrogram of BApNA, *p*-NA, and BA (B).

Fig. S2: Microscopy image of a nanoESI capillary pulled by a P-1000 Sutter instrument. The optimized parameters were as follows: heating temperature of 510 ℃, pulling value of 0, velocity value of 30, delay value of 1, and pressure value of 500.

Fig. S3: Optimization of GA concentration (a), immobilization time (b), and trypsin concentration (c) for the synthesis of Fe_3O_4@CTS-GA-Try.

Fig. S4: TEM images and EDS spectrum of Fe_3O_4 MNPs (A), Fe_3O_4@CTS (B), Fe_3O_4@CTS-GA-Try (C).

Fig. S5: Thermal stability of free trypsin & Fe_3O_4@CTS-GA-Try at 45℃ (a); Effect of temperature (b) and pH value (c) on the activity of free trypsin & Fe_3O_4@CTS-GA-Try

Fig. S6: Mass spectra and recovery of BSA treated by denaturation & reduction & incubation with IAA followed hydrolyzing by Fe_3O_4@CTS-GA-Try.

Fig. S7: Performance of Fe_3O_4@CTS-GA-Try hydrolysis efficiency under different concentration of ACN (A) and the peptides coverage condition under 10% ACN (B).

Fig. S8: Mass spectrum of BSA under optimal conditions.

Fig. S9: Volcano plots of identified peptides comparing free and IMER trypsin digestion.

Fig. S10: Obtained nano-ESI HRMS spectra of abrin protein after hydrolysis by Fe_3O_4@CTS-GA-Try.

Fig. S11: The total ion chromatogram of abrin peptides obtained by nanoESI-HRMS analysis with the acquisition mode of full MS scan coupled with PRM.

Fig. S12: MS/MS spectra of characteristic peptide (GGLIHDIPVLPDPTTLQER) hydrolyzed from abrin. The analysis was performed by nanoESI-MS/MS in positive mode.

Fig. S13: MS/MS spectra of characteristic peptide (FSTEGATSQSYK) hydrolyzed from abrin. The analysis was performed by nanoESI-MS/MS in positive mode.

Fig. S14: MS/MS spectra of characteristic peptides (TLIVIIQMVAEAAR) hydrolyzed from abrin. The analysis was performed by nanoESI-MS/MS in positive mode.

References

Additional Experimental Details

1. Material and reagents

MS grade acetonitrile and formic acid were purchased from Fisher Scientific (Pittsburgh, PA, United States). Ultrapure water used throughout the experiments was produced with a Millipore-Milli-Q® Integral 5 water purification system (Bedford, MA, United States). Ferric trichloride hexahydrate ($FeCl_3 \cdot 6H_2O$), ferrous chloride tetrahydrate ($FeCl_2 \cdot 4H_2O$), and ammonia solution (25% in water) were bought from Shanghai Aladdin Biochemical Technology Co., Ltd. (Shanghai, China). Acetic acid, ammonium bicarbonate (NH_4HCO_3), bovine serum albumin (BSA), trypsin (from bovine pancreas), dithiothreitol (DTT), iodoacetamide (IAA) were purchased from Sigma-Aldrich (Saint Quentin Fallavier, France). Chitosan, sodium hydroxide (NaOH), glutaraldehyde (GA, 25% in water), and N-benzoyl-L-arginine (BA) were obtained from Shanghai Yi En Chemical Technology Co., Ltd. (Shanghai, China). N-benzoyl-DL-arginine-4-nitroanilide hydrochloride (BAPNA) and *p*-nitroaniline (*p*-NA) were purchased from Shanghai Maclin Biochemical Technology Co., Ltd. (Shanghai, China). All of the reagents were of analytical grade. Protein digestion was realized in safe-lock tubes of 1.5 mL from Eppendorf (Hamburg, Germany).

2. Instruments

X-ray powder diffraction (XRD) patterns were collected (0.02°/step, 1°/min) by Rigaku Ultima IV X-ray diffraction (XRD, Rigaku Corporation, Japan) with a Cu Kα X-ray source. Fourier transform infrared (FT-IR) spectra with a spectral scanning range of 4000 − 400 cm^{-1} was acquired by Nicolet iS20 spectrophotometer (Thermo Fisher Scientific Inc., USA). Magnetization curves were measured with a LakeShore7404 vibrating sample magnetometer (VSM, Lake Shore Inc., USA). The morphology and structure were characterized by transmission electron microscopy (TEM) at an operating voltage of 200 kV (FEI, Tecnai F20, USA). Scanning electron microscopy and energy dispersive spectroscopy (EDS) was performed using ZEISS Gemini 300 scanning electron microscope (Carl Zeiss AG, Germany) at an operating voltage of 15 kV. Shimadzu UV-3100 spectrophotometer (Tokyo, Japan) was used for enzyme activity assay. Ultrasonic cell disruptor model HUP-100 (80 W, 20kHz) for ultrasonic probe from Tianjin Hengao Technology Development Co. (Tianjin, China), KQ-100DE ultrasonic bath from Kunshan Ultrasonic Instrument Co. (Kunshan, China), domestic microwave oven, and infrared (IR) lamp from Shanghai Yaming Lighting Co., Ltd. (Shanghai, China) were used to accelerate enzymatic protein digestions. A borosilicate glass tube (1.5 mm o.d., 0.86 mm i.d., 10 cm length) was heated and pulled using a P-1000 Flaming/Brown micropipette puller system (Sutter Instrument, Novato, CA, USA), yielding a tip of approximately 10 μm i.d. (Fig.S2). MS experiments were carried out using a Q-Exactive mass spectrometer (Thermo

Fisher Scientific, Waltham, MA, USA). Further LC-MS/MS data can be obtained by Ultimate 3000 liquid chromatographic system (Thermo Fisher Scientific, Waltham, MA, USA) coupled to Q-Exactive mass spectrometer.

3. Activity assays of free trypsin and Fe_3O_4@CTS-GA-Try

Photometric assay for verification of trypsin activity based on the method reported before[1]. The enzymatic activity of free trypsin was measured by hydrolysis of 0.5 mM BAPNA solution in 20 mmol/L NH_4HCO_3 buffer solution for 5 min at room temperature. The absorbance was measured at 410 nm every 30 s for 3 min at 37 °C and the rate of formation of *p*-NA is directly proportional to the trypsin catalytic activity. The enzymatic activity of Fe_3O_4@CTS-GA-Try was measured by hydrolysis of 0.5 mM BAPNA for 3 min at room temperature in an orbital shaker. After incubation, immobilized trypsin was separated by magnetic decantation. Each experiment was carried out three times in parallel, and the average of the absorbance was taken for statistical analysis of catalytic capacity. The maximum enzyme activity in each group was set to be 100%, and the activity contrast with it was the relative activity. One unit of trypsin activity was defined as 1 μmol of *p*-NA released per minute, using 8800 $L \cdot mol^{-1} \cdot cm^{-1}$ as the extinction coefficient of *p*-NA at 410 nm.

4. Michaelis-Menten constants of free and immobilized trypsin

The Michaelis constant (Km) & the maximum reaction rate (Vmax) of the free and immobilized trypsin were investigated at different substrate concentrations (S) of BAPNA (0.05 mM−1mM) in the 20 mmol/L NH_4HCO_3 buffer solution at 25 ℃ and calculated the values from Lineweaver-Burk Plots using the initial rate of the enzymatic reaction. The Michaelis-Menten equation is interpreted as follows:

$$\frac{1}{V} = \frac{K_m}{V_{max}} \times \frac{1}{[S]} + \frac{1}{V_{max}}$$

Where $[S]$ is the concentration of the substrate, V is the initial reaction rate, V_{max} is the maximum rate of the reaction, and K_m is the Michaelis constant.

5. UHPLC-HRMS anlyisis

The UHPLC separation was performed using an UltiMate 3000 liquid chromatograph equipped with a quaternary solvent pump, an online degasser, a PAL autosampler (CTC Analytics, Zwingen, Switzerland), and a thermostatted column compartment (Thermo Scientific, Sunnyvale, CA, United States). Chromatographic separation was conducted on a Waters ACQUITY UPLC Peptide BEH C18 analytical column (130 Å, 2.1 mm × 150 mm, 1.7 μm) at a flow rate of 0.25 mL/min. The column temperature was maintained at 30 ℃. A sampling volume of 2 μL was injected for analysis. The mobile phase consisted of 0.1% (*v*/*v*) formic acid solution as

the aqueous phase (A) and 0.1 (*v*/*v*) formic acid in acetonitrile as the organic phase (B) using a gradient elution program. The elution conditions were set as follows: 0–5 min, 5% B; 5–20 min, 5%–60% B; 20–30 min, 60%–95% B; 30–32 min, 95% B; 32–35 min, 95%–5% B, 35–37 min, 5% B.

HRMS analysis was performed on a benchtop Q-Exactive hybrid quadrupole-Orbitrap mass spectrometer coupled with an electrospray ionization (ESI) source operated in positive ion mode. The analysis was performed by the full-scan MS/data-dependent MS/MS (full-scan MS^1/dd-MS^2) acquisition mode. Source parameters were set as follows: spray voltage, 3.5 kV; sheath gas pressure, 40 arb; auxiliary gas pressure, 10 arb; sweep gas pressure, 0 arb; capillary temperature, 350 ℃; and auxiliary gas heater temperature, 320 °C. The analyzer scanned within the range of *m/z* 150–2000 at the resolution of 70000 full width at half maximum (FWHM) in the full scan mode. Automatic gain control (AGC) target value was set at 1×10^6 with a maximum injection time (IT) of 100 ms. The dd-MS2 confirmation mode was conducted at a mass resolution of 17500 FWHM using an isolation window of 3.0 *m/z*. User-defined top 5 ion selection was initiated by an intensity threshold of 1×10^5. Three collision energy steps were applied at 20%, 30%, and 40%.

Supplementary Tables:

Table S1. Annotated peptides found under the optimal digestion conditions of BSA.

Start	End	Sequence	Observed ions	z
20	24	r.GVFRR.d	634.318	1
20	28	r.GVFRRDTHK.s	372.9499	3
24	34	r.RDTHKSEIAHR.f	675.277	2
25	28	r.DTHK.s	500.3524	1
29	34	k.SEIAHR.f	356.7212	2
29	36	k.SEIAHRFK.d	330.1952	3
35	44	r.FKDLGEEHFK.g	1249.4312, 625.2823,417.2105	1, 2, 3
37	44	k.DLGEEHFK.g	487.7306, 325.4923	2, 3
66	75	k.LVNELTEFAK.t	1163.4342, 582.2532	1, 2
76	88	k.TCVADESHAGCEK.s	675.277	2
89	100	k.SLHTLFGDELCK.v	681.6842, 454.8934	2, 3
101	105	k.VASLR.e	545.3374	1
118	130	k.QEPERNECFLSHK.d	539.5806	3
123	130	r.NECFLSHK.d	326.6729	3
131	138	k.DDSPDLPK.l	886.4139, 443.7102	1, 2
156	160	k.KFWGK.y	665.3386, 333.1943	1, 2
157	160	k.FWGK.y	537.2801	1
161	167	k.YLYEIAR.r	464.2514	2
161	168	k.YLYEIARR.h	542.2662	2
205	209	k.IETMR.e	649.2982, 325.1725	1, 2
205	211	k.IETMREK.v	454.1949	2
205	218	k.IETMREKVLASSAR.q	530.9198	3
212	220	k.VLASSARQR.l	494.7847, 330.1952	2, 3
219	228	r.QRLRCASIQK.f	401.1981	3
223	228	r.CASIQK.f	649.2982, 325.1725	1, 2
223	232	r.CASIQKFGER.a	569.4254	2
229	232	k.FGER.a	508.2526	1
233	241	r.ALKAWSVAR.l	501.7917	2
236	241	k.AWSVAR.l	689.3212, 345.1922	1, 2
236	248	k.AWSVARLSQKFPK.a	759.2993, 506.5876	2, 3
242	248	r.LSQKFPK.a	847.3984, 423.9653	1, 2
246	248	k.FPK.a	391.1805	1
246	256	k.FPKAEFVEVTK.l	432.2405	3
249	256	k.AEFVEVTK.l	922.3711, 461.6759	2
249	263	k.AEFVEVTKLVTDLTK.v	564.94	3
257	263	k.LVTDLTK.v	789.3911, 395.2409	1, 2
281	285	r.ADLAK.y	517.3147	1
286	297	k.YICDNQDTISSK.l	693.7283	2
300	309	k.ECCDKPLLEK.s	589.7749	2
310	318	k.SHCIAEVEK.d	1015.3626, 507.8146	1, 2

347	359	k.DAFLGSFLYEYSR.r	784.8189, 523.6009	2, 3
347	360	k.DAFLGSFLYEYSRR.h	574.8992	3
360	371	r.RHPEYAVSVLLR.l	720.3534, 480.6087	2, 3
361	371	r.HPEYAVSVLLR.l	642.3245, 428.5762	2, 3
375	386	k.EYEATLEECCAK.d	694.3088, 463.5789	2, 3
375	401	k.EYEATLEECCAKDDPHAC YSTVFDKLK.h	1036.6008	3
400	412	k.LKHLVDEPQNLIK.q	516.303	3
402	412	k.HLVDEPQNLIK.q	1305.5155, 653.3258, 435.5524	1, 2, 3
413	420	k.QNCDQFEK.l	506.2142	2
421	433	k.LGEYGFQNALIVR.y	1479.6161, 740.3424, 493.9351	1, 2, 3
434	437	r.YTRK.v	567.3711	1
437	451	r.KVPQVSTPTLVEVSR.s	820.3871, 547.3141	2, 3
438	451	k.VPQVSTPTLVEVSR.s	756.3508, 504.6155	2, 3
438	455	k.VPQVSTPTLVEVSRSLGK.v	633.0015	3
452	455	r.SLGK.v	404.2298	1
452	459	r.SLGKVGTR.c	817.0006, 409.7158	1, 2
460	468	r.CCTKPESER.m	526.7857	2
483	489	r.LCVLHEK.t	421.6931	2
490	498	k.TPVSEKVTK.c	494.7847, 330.1952	2, 3
496	507	k.VTKCCTESLVNR.r	451.2314	3
499	507	k.CCTESLVNR.r	1024.4136	1
508	523	r.RPCFSALTPDETYVPK.a	608.2817	3
524	528	k.AFDEK.l	609.2571, 305.1498	1, 2
545	557	k.QIKKQTALVELLK.h	756.3508, 504.6155	2, 3
548	557	k.KQTALVELLK.h	1142.5213, 571.8450, 381.5758	1, 2, 3
549	557	k.QTALVELLK.h	1014.4702, 507.8146	1, 2
562	568	k.ATEEQLK.t	818.3014, 409.7158	1, 2
569	580	k.TVMENFVAFVDK.c	700.2598	2
588	597	k.EACFAVEGPK.l	525.2591	2
598	607	k.LVVSTQTALA.	1002.4353, 501.7917	1, 2

Table S2. Abrin-a (Uniport P11140) measured *m/z* values of trypsin digest peptides found for the digestion of abrin. Mass tolerance was set to ±10 ppm.

Slice	Amino acid sequence	$[M+H]^{1+}$	$[M+H]^{2+}$	$[M+H]^{3+}$	$[M+H]^{4+}$	$[M+H]^{5+}$	$[M+H]^{1+}$
[1-6]	.QDRPIK.f	756.4319	378.7246	□	□	□	□
[7-18]	**k.FSTEGATSQSYK.q**	□	**653.3042**	□	327.1508	□	□
[19-25]	**k.QFIEALR.e**	876.4908	**438.7546**	□	□	□	□
[26-27]	r.ER.l	304.1631	□	□	□	□	□
[28-48]	r.LRGGLIHDIPVLPDPTTLQER.n	□	□	780.7761	585.8305	□	□
[30-48]	r.GGLIHDIPVLPDPTTLQER.n	□	1036.063	□	□	□	□
[30-50]	**r.GGLIHDIPVLPDPTTLQERNR.y**	□	□	**691.0433**	586.0705	□	□
[89-118]	**r.DAPSSASDYLFTGTDQHSLPFYGTYGDLER.w**	□	□	**1104.162**	828.3783	□	□
[119-124]	**r.WAHQSR.q**	784.3883	**392.6974**	□	□	□	□
[125-142]	**r.QQIPLGLQALTHGISFFR.s**	□	□	**676.0481**	□	□	□
[143-151]	r.SGGNDNEEK.a	□	□	317.1339	□	□	□
[143-153]	**r.SGGNDNEEKAR.t**	□	**588.7617**	□	□	□	□
[154-167]	**r.TLIVIIQMVAEAAR.f**	□	**764.4531**	509.971	382.723	□	□
[168-169]	r.FR.y	□	□	□	□	□	□
[168-174]	r.FRYISNR.v	955.5127	478.2541	□	□	□	□
[170-174]	r.YISNR.v	652.3429	326.6763	□	□	□	□
[170-176]	r.YISNRVR.v	907.515	454.2543	□	□	□	□
[177-203]	**r.VSIQTGTAFQPDAAMISLENNWDNLSR.g**	□	□	**993.4831**	745.3649	□	□
[204-223]	**r.GVQESVQDTFPNQVTLTNIR.n**	□	□	**749.3816**	□	□	□
[266-272]	k.SKICSSR.y	□	390.7069	□	□	□	□
[268-272]	k.ICSSR.y	565.2786	□	□	□	□	□
[268-278]	k.ICSSRYEPTVR.i	□	655.823	□	□	□	□
[273-278]	**r.YEPTVR.i**	764.3931	**382.7028**	□	□	□	□
[273-282]	r.YEPTVRIGGR.d	□	574.3144	383.2179	□	□	□
[279-282]	r.IGGR.d	402.2404	□	□	□	□	□
[300-304]	r.IIMWK.c	□	345.7064	□	□	□	□
[300-306]	r.IIMWKCK.d	□	461.2573	□	□	□	□
[305-308]	k.CKDR.l	521.256	□	□	□	□	□

[307-318]	k.DRLEENQLWTLK.s	□	772.905	515.6031	□	□	□
[309-318]	r.LEENQLWTLK.s	1273.677	637.3452	425.2383	□	□	□
[319-321]	k.SDK.t	349.1749	□	□	□	□	□
[319-324]	k.SDKTIR.s	719.4054	360.2054	□	□	□	□
[322-324]	k.TIR.s	389.252	□	□	□	□	□
[322-328]	k.TIRSNGK.c	□	388.2264	□	□	□	□
[395-398]	r.QGWR.t	546.2735	□	□	□	□	□
[395-434]	r.QGWRTGNNTSPFVTSISGYSDLCMQAQGSNVWMADCDSNK.k					871.9879	726.8266
[436-448]	k.EQQWALYTDGSIR.s	□	783.881	522.9263	□	□	□
[461-463]	k.DHK.q	399.1999		□	□	□	□
[464-481]	k.QGSTILLMGCSNGWASQR.w	□	954.9544	□	□	□	□
[482-485]	r.WVFK.n	579.328	□	□	□	□	□
[503-509]	k.GSDPSLK.q	703.3626	352.1876	□	□	□	□
[510-528]	**k.QIILWPYTGKPNQIWLTLF.**	□	□	**777.7698**	583.5757	□	□

Table S3. Abrin-b (Uniport Q06077) measured *m/z* values of trypsin digest peptides found for the digestion of abrin. Mass tolerance was set to ±10 ppm.

Slice	Amino acid sequence	$[M+H]^{1+}$	$[M+H]^{2+}$	$[M+H]^{3+}$	$[M+H]^{4+}$	$[M+H]^{5+}$
[1-6]	.QDQVIK.f	730.4011	365.7073			
[7-18]	**k.FTTEGATSQSYK.q**	**660.3006**				
[7-25]	k.FTTEGATSQSYKQFIEALR.q				545.028	436.2231
[19-25]	**k.QFIEALR.q**	876.4908	**438.7545**			
[26-27]	r.QR.l	303.1794				
[28-48]	**r.LTGGLIHGIPVLPDPTTLQER.n**			**743.0883**		
[80-88]	r.AGNRSYFLR.d		542.2861			
[84-88]	r.SYFLR.d		343.1804			
[89-96]	r.DAPTSASR.y		402.6905			
[97-108]	r.YLFTGTQQYSLR.f		738.8768	492.9285		
[109-118]	r.FNGSYIDLER.l	565.2787				
[119-124]	r.LARQTR.q	744.4497	372.722			
[125-135]	**r.QQIPLGLQALR.h**		**618.8741**	412.917		
[125-152]	**r.QQIPLGLQALRHAISFLQSGTDDQEIAR.t**				777.1605	**621.9392**
[136-152]	**r.HAISFLQSGTDDQEIAR.t**			**629.9776**		
[153-166]	**r.TLIVIIQMASEAAR.y**		**758.4345**	505.9579		
[153-168]	**r.TLIVIIQMASEAARYR.f**		**918.0184**	612.3437		
[167-168]	r.YR.f	338.1827				
[167-173]	r.YRFISYR.v		502.7651	335.5155		
[169-173]	r.FISYR.v		343.1804			
[169-179]	r.FISYRVGVSIR.t			432.9168		
[174-179]	**r.VGVSIR.t**	630.3938	**315.7051**			
[265-271]	k.SKICSSR.y		390.707			
[267-277]	k.ICSSRYEPTVR.i		655.8272			
[272-277]	**r.YEPTVR.i**	764.3929	**382.7028**			
[272-281]	r.YEPTVRIGGR.n		574.3145	383.2185		
[278-281]	r.IGGR.n	402.2504				
[299-303]	r.IIAWK.c	630.3939	310.7051			

[299-305]	r.IIAWKCK.d		431.2535	
[304-307]	k.CKDR.l	521.256		
[306-317]	k.DRLEENQLWTLK.s		772.9050	515.6031
[308-317]	r.LEENQLWTLK.s	1273.677	637.3452	425.2383
[318-320]	k.SDK.t	349.1749		
[318-323]	k.SDKTIR.s	719.4054	360.2054	
[321-323]	k.TIR.s	389.252		
[321-327]	k.TIRSNGK.c		388.2264	
[394-397]	r.QGWR.t	546.2735		
[435-447]	k.EQQWALYTDGSIR.s		783.8810	522.9263
[460-462]	k.DHK.q	399.1999		
[502-508]	k.RSDPSLK.e	802.4443		
[503-508]	r.SDPSLK.e	646.3422		

Table S4. Abrin-c (Uniport P28590) measured *m/z* values of trypsin digest peptides found for the digestion of abrin. Mass tolerance was set to ±10 ppm.

Slice	Amino acid sequence	$[M+H]^{1+}$	$[M+H]^{2+}$	$[M+H]^{3+}$	$[M+H]^{4+}$	$[M+H]^{5+}$
[1-3]	.MDK.t	393.182				
[1-6]	.MDKTLK.l	735.4001				
[4-6]	k.TLK.l	361.2167				
[7-22]	**k.LLILCLAWTCSFSALR.c**		**905.4998**	603.9925		
[41-52]	**k.FTTEGATSQSYK.q**		**660.3076**			
[41-59]	k.FTTEGATSQSYKQFIEALR.q				545.028	436.2231
[53-59]	k.QFIEALR.q	876.4908	438.7545			
[60-61]	r.QR.l	303.1794				
[62-82]	**r.LTGGLIHDIPVLPDPTTVEER.n**		1136.618	**758.0757**		
[83-95]	r.NRYITVELSNSER.e		790.9074	527.6051		
[85-95]	**r.YITVELSNSER.e**		**655.8372**			
[85-113]	r.YITVELSNSERESIEVGIDVTNAYVVAYR.a				823.1703	658.7332
[114-122]	r.AGSQSYFLR.d		514.7699	343.5199		
[123-138]	r.DAPASASTYLFPGTQR.y				421.2118	
[139-152]	r.YSLRFDGSYGDLER.w		839.3996			
[143-152]	**r.FDGSYGDLER.w**		**579.7547**	386.8445		
[159-176]	**r.EEISLGLQALTHAISFLR.s**			**666.7007**		
[188-201]	**r.TLIVIIQMASEAAR.y**		**758.4345**	505.9579		
[188-203]	**r.TLIVIIQMASEAARYR.y**		**918.0184**			
[202-203]	r.YR.y	338.1827				
[202-208]	r.YRYISNR.v	974.5056	486.2596			
[204-208]	r.YISNR.v	652.3424	326.6758			
[209-214]	**r.VGVSIR.t**	630.3938	**315.7051**			
[295-301]	r.SIVEESK.i		396.2198			
[295-306]	r.SIVEESKICSSR.y			446.5684		
[302-306]	k.ICSSR.y	565.2787				
[302-312]	k.ICSSRYEPTVR.i		655.8272			
[307-312]	r.YEPTVR.i	764.3929	382.7028			

[307-316]	r.YEPTVRIGGR.d		574.3145	383.2185	
[313-316]	r.IGGR.d	402.2404			
[334-338]	r.IIAWK.c	630.3939	315.7051		
[334-340]	r.IIAWKCK.d		431.2535		
[339-342]	k.CKDR.l	521.256			
[341-352]	k.DRLEENQLWTLK.s		772.9050	515.6031	
[343-352]	r.LEENQLWTLK.s	1273.677	637.3152	425.2383	
[353-355]	k.SDK.t	349.1749			
[353-358]	k.SDKTIR.s	719.4054	360.2054		
[356-358]	k.TIR.s	389.252			
[356-362]	k.TIRSNGK.c		388.2264		
[429-432]	r.QGWR.t	546.2735			
[433-468]	r.TGNNTSPFVTSISGYSDLCMQAQGSNVWLADCDNNK.k				768.3486
[470-482]	k.EQQWALYTDGSIR.s		783.8364	522.9263	
[495-497]	k.DHK.q	399.1999			
[516-519]	r.WLFK.n	593.3421			
[537-543]	k.RSDPSLK.e	802.4443			
[538-543]	r.SDPSLK.e	646.3422			

Table S5. Abrin-d (Uniport Q06076) measured *m/z* values of trypsin digest peptides found for the digestion of abrin. Mass tolerance was set to ±10 ppm.

Slice	Amino acid sequence	$[M+H]^{1+}$	$[M+H]^{2+}$	$[M+H]^{3+}$	$[M+H]^{4+}$	$[M+H]^{5+}$
[1-6]	.QDQVIK.f	730.4011	365.7073	□	□	□
[7-18]	**k.FTTEGATSQSYK.q**	□	660.3076	□	□	□
[7-25]	k.FTTEGATSQSYKQFIEALR.q	□	□	□	545.028	436.2231
[19-25]	**k.QFIEALR.q**	876.4908	**438.7545**	□	□	□
[26-27]	r.QR.l	303.1794	□	□	□	□
[28-48]	**r.LTGGLIHDIPVLPDPTTVEER.n**	□	1136.6179	**758.0757**	□	□
[49-61]	r.NRYITVELSNSER.e	□	790.9074	527.6051	□	□
[51-61]	**r.YITVELSNSER.e**	□	**655.8372**	□	□	□
[51-79]	r.YITVELSNSERESIEVGIDVTNAYVVAYR.a	□	□	1097.228	823.1703	658.7332
[80-88]	r.AGSQSYFLR.d	□	514.7699	343.5199	□	□
[89-104]	r.DAPASASTYLFPGTQR.y	□	□	□	421.2118	□
[105-118]	r.YSLRFDGSYGDLER.w	□	839.8096	□	□	□
[109-118]	**r.FDGSYGDLER.w**	□	**579.7547**	386.8445	□	□
[125-142]	**r.EEISLGLQALTHAISFLR.s**	□	□	**666.7007**	□	□
[154-167]	**r.TLIVIIQMASEAAR.y**	□	**758.4345**	505.9579	□	□
[154-169]	**r.TLIVIIQMASEAARYR.c**	□	**918.0184**	□	□	□
[168-169]	r.YR.c	338.1827	□	□	□	□
[168-174]	r.YRCISNR.v	□	□	304.4869	□	□
[170-180]	**r.CISNRVGVSIR.t**	1203.6644	602.3393	**401.8907**	□	□
[175-180]	**r.VGVSIR.t**	630.3938	**315.7051**	□	□	□
[261-267]	r.SIVEESK.i	□	396.2198	□	□	□
[261-272]	r.SIVEESKICSSR.y	□	□	446.5684	□	□
[268-272]	k.ICSSR.y	565.2787	□	□	□	□
[268-278]	k.ICSSRYEPTVR.i	□	655.8272	□	□	□
[273-278]	**r.YEPTVR.i**	764.3929	**382.7028**	□	□	□
[273-282]	r.YEPTVRIGGR.d	□	574.3145	383.2185	□	□
[279-282]	r.IGGR.d	402.2404	□	□	□	□
[300-304]	r.IIAWK.c	630.3939	315.7051	□	□	□

[300-306]	r.IIAWKCK.d	□	431.2535	□	□	□
[305-308]	k.CKDR.l	521.256	□	□	□	□
[307-318]	k.DRLEENQLWTLK.s	□	772.9050	515.6031	□	□
[309-318]	r.LEENQLWTLK.s	1273.6766	637.3452	425.2383	□	□
[309-324]	r.LEENQLWTLKSDLTIR.s	□	980.0396	653.6840	490.512	□
[319-324]	k.SDLTIR.s	704.3922	□	□	□	□
[395-398]	r.QGWR.t	546.2735	□	□	□	□
[399-434]	r.TGNNTSPFVTSISGYSDLCMQAQGSNVWLADCDNNK.k	□	□	□	□	768.3486
[436-448]	k.EQQWALYTDGSIR.s	□	783.8364	522.9263	□	□
[461-463]	k.DHK.q	399.1999	□	□	□	□
[482-485]	r.WLFK.n	593.3421	□	□	□	□
[503-509]	k.GSDPSLK.q	703.3626	352.1876	□	□	□
[510-528]	**k.QIILWPYTGKPNQIWLTLF.**	□	□	**777.7698**	583.5757	□

Table S6. Agglutinin-1 (Uniport Q9M6E9) measured *m/z* values of trypsin digest peptides found for the digestion of abrin. Mass tolerance was set to ±10 ppm.

Slice	Amino acid sequence	[M+H] $^{1+}$	[M+H] $^{2+}$	[M+H] $^{3+}$	[M+H] $^{4+}$	[M+H] $^{5+}$
[1-7]	.MKFETTK.n		442.7394			
[3-7]	k.FETTK.n	625.3174				
[26-44]	**k.FTTGSATPASYNQFIDALR.e**		1030.503	**687.3401**		
[26-46]	k.FTTGSATPASYNQFIDALRER.l		1173.081	782.3814	587.0428	
[45-46]	r.ER.l	304.163				
[45-59]	r.ERLTGGLIYGIPVLR.d		828.9972			
[47-59]	**r.LTGGLIYGIPVLR.d**		**686.4272**			
[60-98]	r.DPSTVEKPNQYVTVELSYSDTVSIQLGIDLTNAYVVAYR.a				1087.803	870.4478
[99-107]	**r.AGSESFFFR.n**		**524.248**			
[138-143]	**k.WAHQSR.q**	784.3874	**392.6978**			
[138-145]	k.WAHQSRQR.i		534.7771			
[144-145]	r.QR.i	303.1794				
[144-154]	**r.QRISLGLEALR.q**		628.375	**419.2571**		
[146-154]	**r.ISLGLEALR.q**	971.586	**486.2981**			
[146-158]	r.ISLGLEALRQGIK.f		699.4219	466.6292		
[155-158]	r.QGIK.f	445.2704				
[155-161]	r.QGIKFLR.s		431.2636			
[159-161]	k.FLR.s	435.276				
[159-172]	k.FLRSGASDDEEIAR.t		783.381	522.5963		
[162-172]	**r.SGASDDEEIAR.t**	1149.502	**575.254**	383.8385		
[162-186]	r.SGASDDEEIARTLIVIIQMVAEAAR.f		1329.732	886.7996	665.3472	
[173-186]	**r.TLIVIIQMVAEAAR.f**		**764.4531**	509.9603		
[187-188]	r.FR.y	322.1898				

[187-192]	**r.FRYVSK.l**		**400.2272**	
[189-192]	r.YVSK.l	496.2758		
[189-201]	**r.YVSKLVVISLSNR.a**		739.4469	**493.2985**
[193-201]	**k.LVVISLSNR.a**	1000.615	**500.8196**	
[202-222]	**r.AAFQPDPSMLSLENTWEPLSR.a**		1195.072	**797.0509**
[243-245]	r.QER.v	432.222		
[280-286]	r.SVVEQSK.i	776.4191	388.7136	
[298-301]	r.IGGR.d	402.2404		
[338-340]	k.SDK.t	349.1749		
[338-343]	k.SDKTIR.s	719.9054	360.2054	
[341-343]	k.TIR.s	389.252		
[341-345]	k.TIRSK.g	604.3771		
[388-407]	**k.SGLVLSAESSSMGGTLTVQK.n**		976.5083	**651.3313**
[408-411]	k.NDYR.m	567.256		
[408-413]	k.NDYRMR.q	854.3917		
[412-413]	r.MR.q	306.1512		
[412-417]	r.MRQGWR.t	833.4196		
[414-417]	r.QGWR.t	546.2735		
[418-434]	r.TGNDTSPFVTSIAGFFK.l		894.9473	
[483-500]	k.QGATIVMMGCSNAWASQR.w		955.9373	
[501-504]	r.WVFK.s	579.328		
[505-521]	**k.SDGTIYNLYDDMVMDVK.s**		989.9410	**660.2905**
[522-528]	**k.SSDPSLK.q**	733.3754	**367.1942**	
[529-547]	k.QIILWPYTGNANQMWATLF.		1134.079	

Table S7. Comparison between the method reported in this work and previously reported methods for abrin identification.

NO.	Preparation before digestion	Digestion process	Analytical method	Total analysis time	Ref.
1	Denatured at 95 ℃ for 15 min	Bath-type sonic at 37 ℃ for 30 min with trypsin	HPLC-MS/MS	More than 2 h	[2]
2	Denatured at 95 ℃ for 10 min and 30 min at 37 ℃ for alkylation	Overnight incubation at 37 ℃ with trypsin	NanoLC-MS/MS	More than 16 h	[3]
3	Denatured at 95 ℃ for 15 min	Ultrasound water bath at 45 ℃ for 15 min with trypsin in 10% ACN	HPLC-MS/MS	More than 50 min	[4]
4	Dryness at 70 °C under a mild nitrogen flow	Digested at 40 °C for 2 h with trypsin	HPLC-MS/MS	More than 1 h	[5]
This work	Denatured at 95 ℃ for 5 min	Ultrasound probe-assisted 15 s with IMER	Direct nano-ESI-MS	Less than 10 min	–

Supplementary Figures:

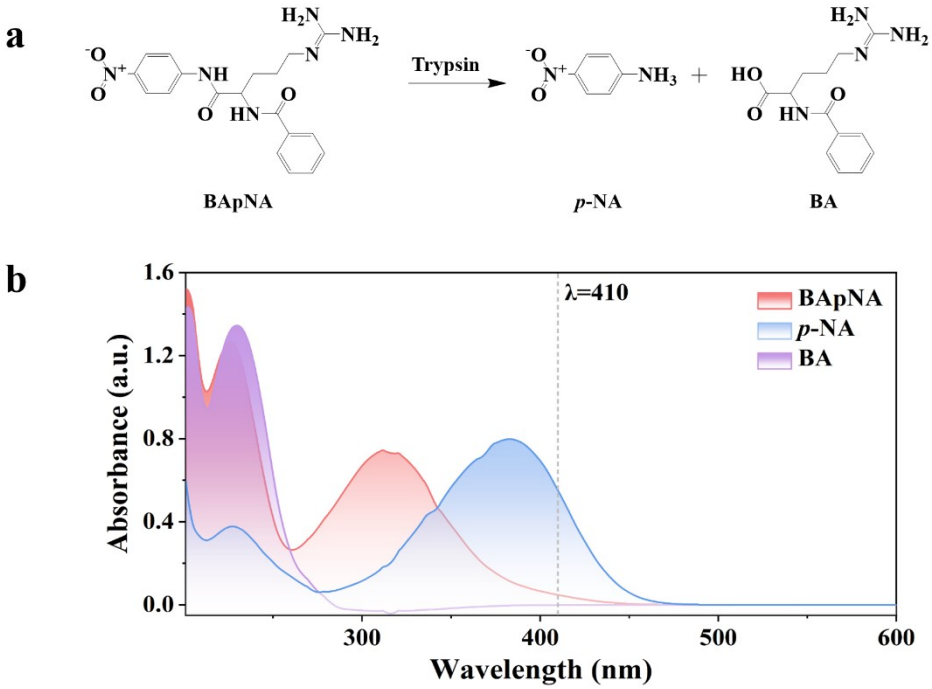

Fig. S1. Reaction equation of trypsin-catalyzed hydrolysis of BApNA (a) and ultraviolet spectrogram of BApNA, *p*-NA, and BA (b).

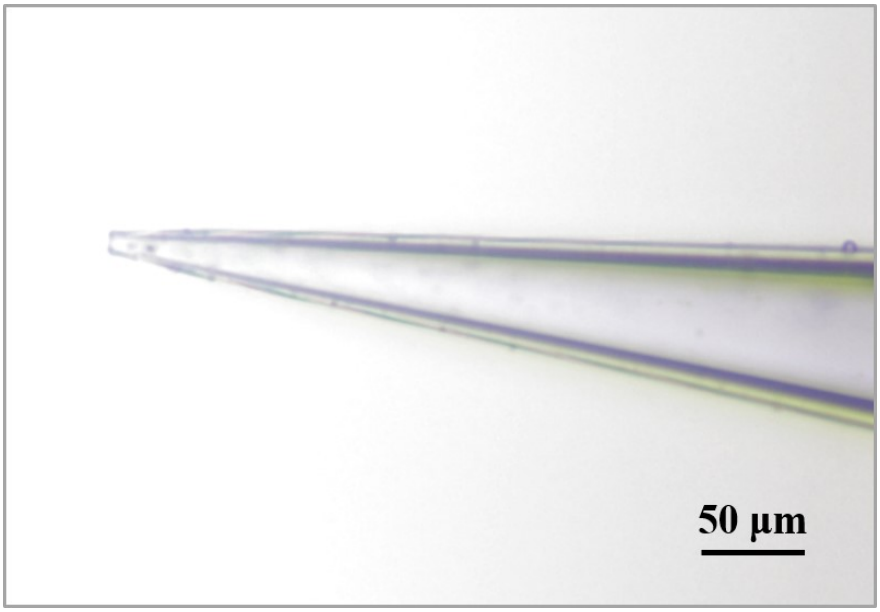

Fig. S2. Microscopy image of a nanoESI capillary pulled by a P-1000 Sutter instrument. The optimized parameters were as follows: heating temperature of 510 °C, pulling value of 0, velocity value of 30, delay value of 1, and pressure value of 500.

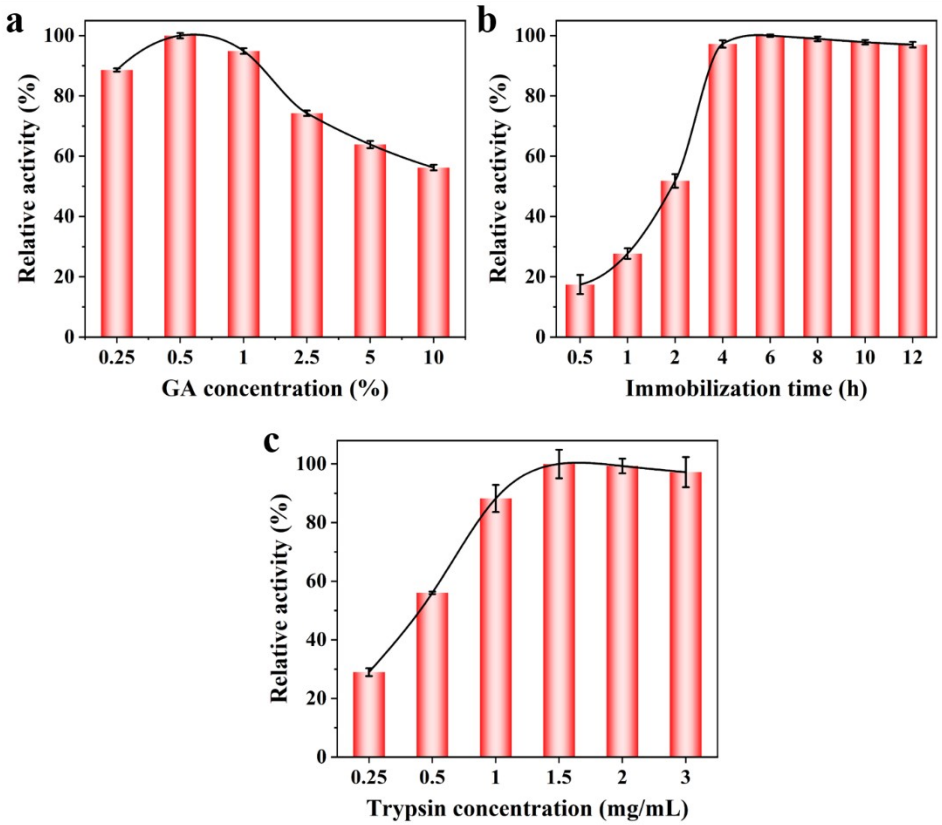

Fig. S3. Optimization of GA concentration (a), immobilization time (b), and trypsin concentration (c) for the synthesis of Fe_3O_4@CTS-GA-Try.

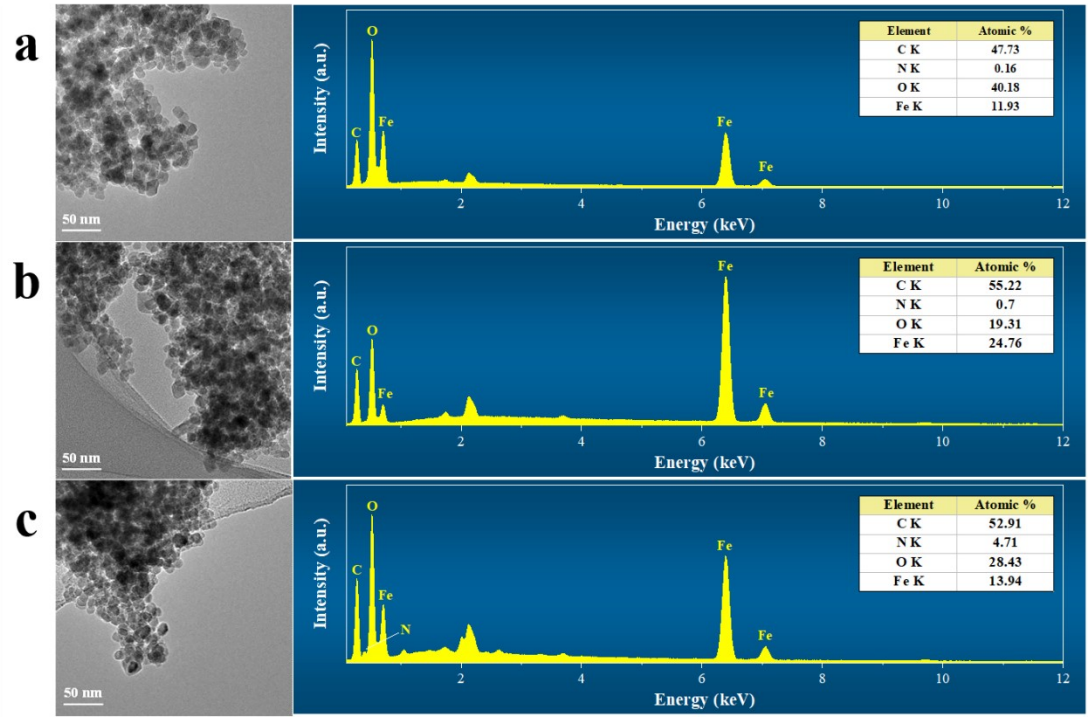

Fig. S4. TEM images and EDS spectrum of Fe_3O_4 MNPs (a), Fe_3O_4@CTS (b), Fe_3O_4@CTS-GA-Try (c).

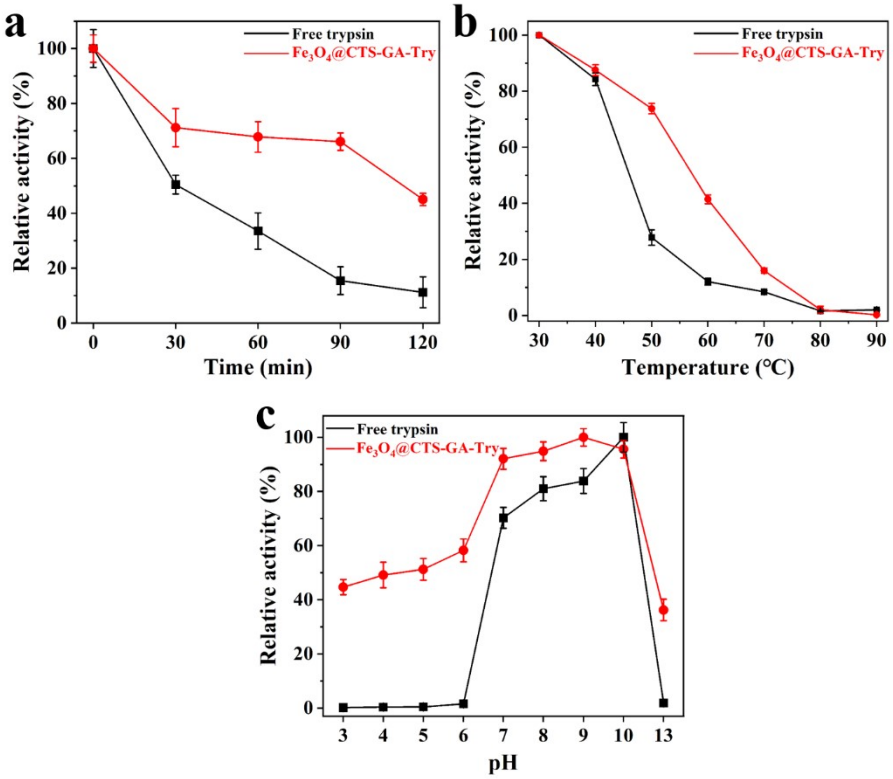

Fig. S5. Thermal stability of free trypsin & Fe_3O_4@CTS-GA-Try at 45℃ (a); Effect of temperature (b) and pH value (c) on the activity of free trypsin & Fe_3O_4@CTS-GA-Try.

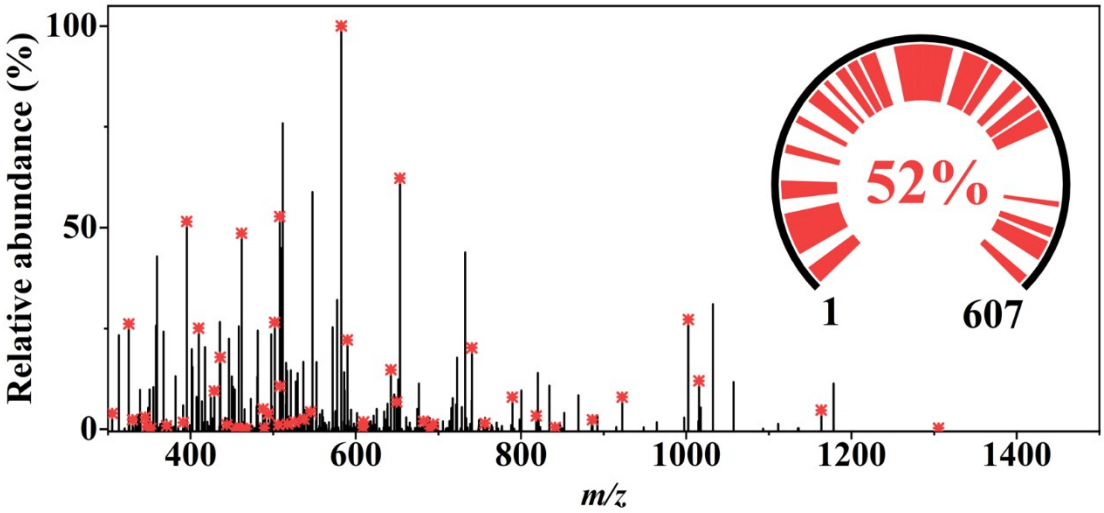

Fig. S6. Mass spectra and recovery of BSA treated by denaturation & reduction & incubation with IAA followed hydrolyzing by Fe_3O_4@CTS-GA-Try.

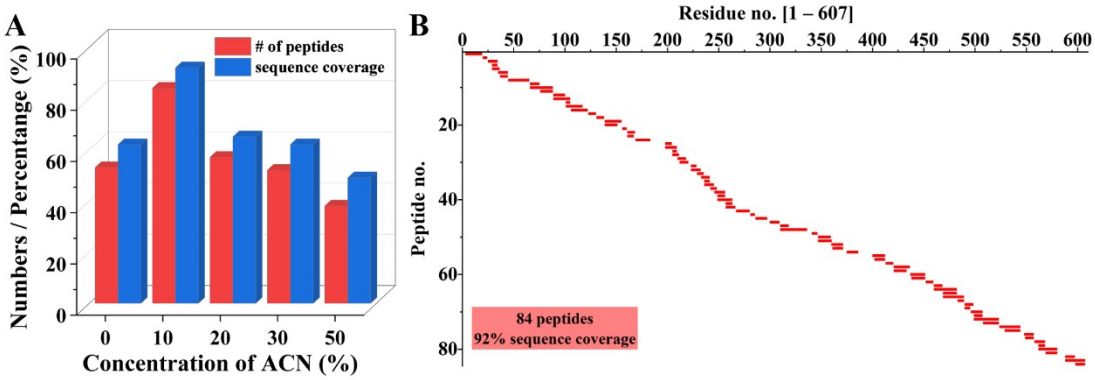

Fig. S7. Performance of Fe_3O_4@CTS-GA-Try hydrolysis efficiency under different concentration of acetonitrile (A) and the peptides coverage condition under 10% acetonitrile (B).

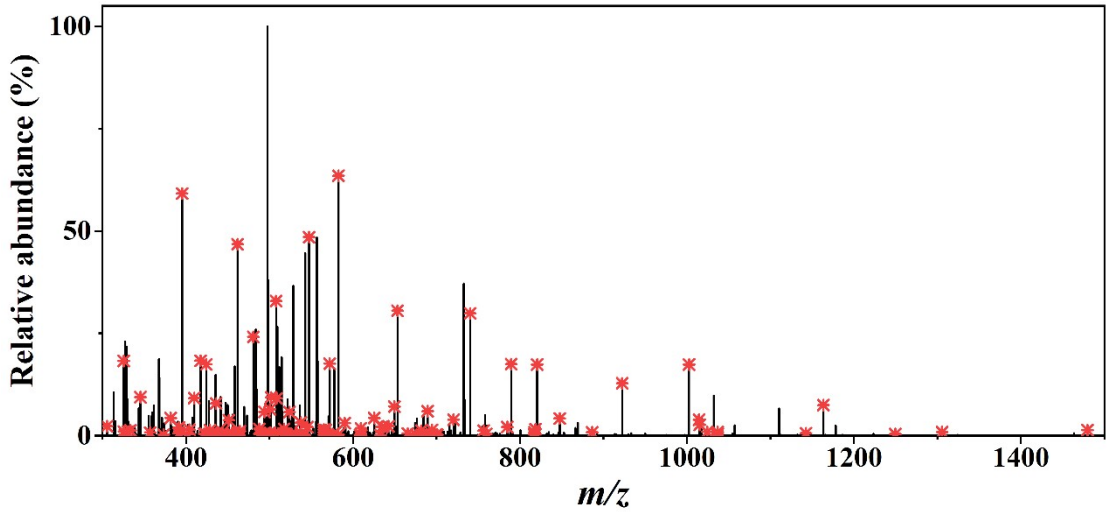

Fig. S8. Mass spectrum of BSA under optimal conditions.

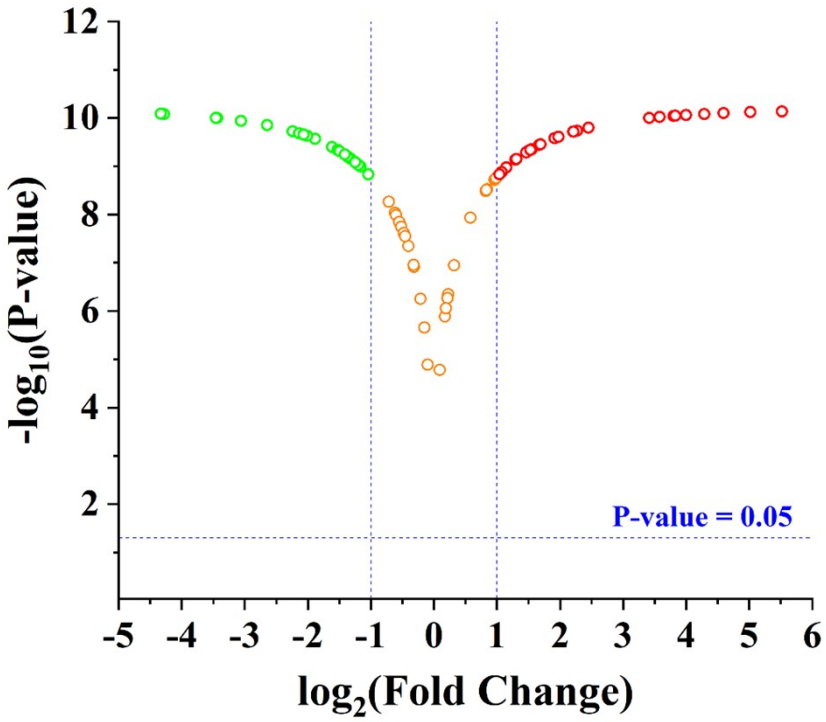

Fig. S9. Volcano plots of identified peptides comparing free and IMER trypsin digestion.

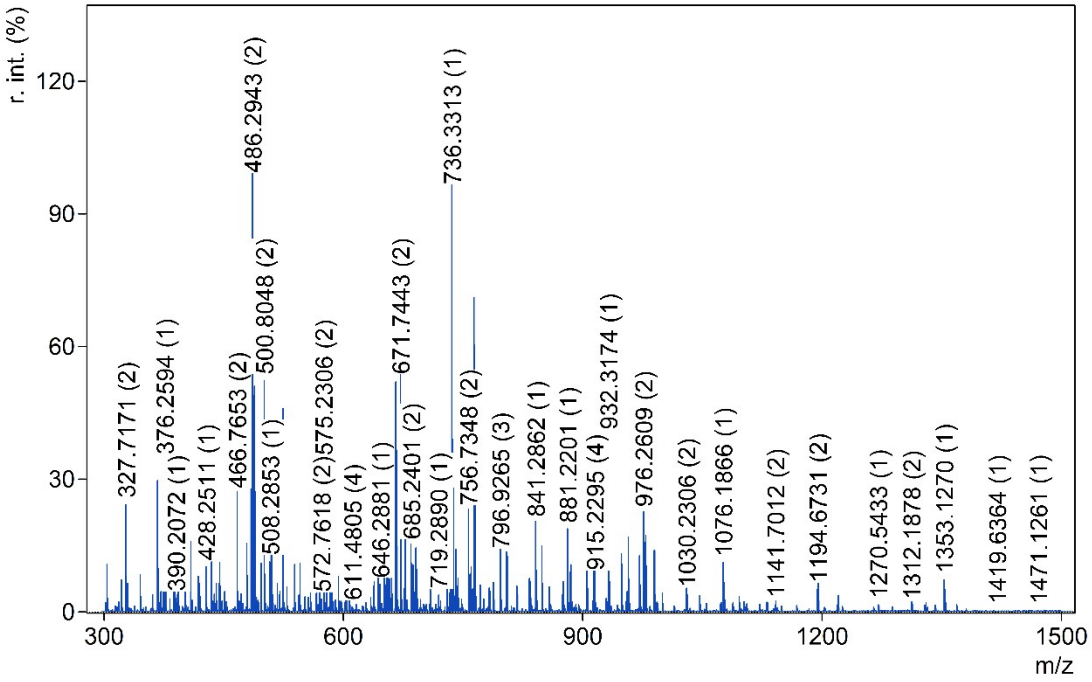

Fig. S10. Obtained nano-ESI HRMS spectra of abrin protein after hydrolysis by Fe_3O_4@CTS-GA-Try.

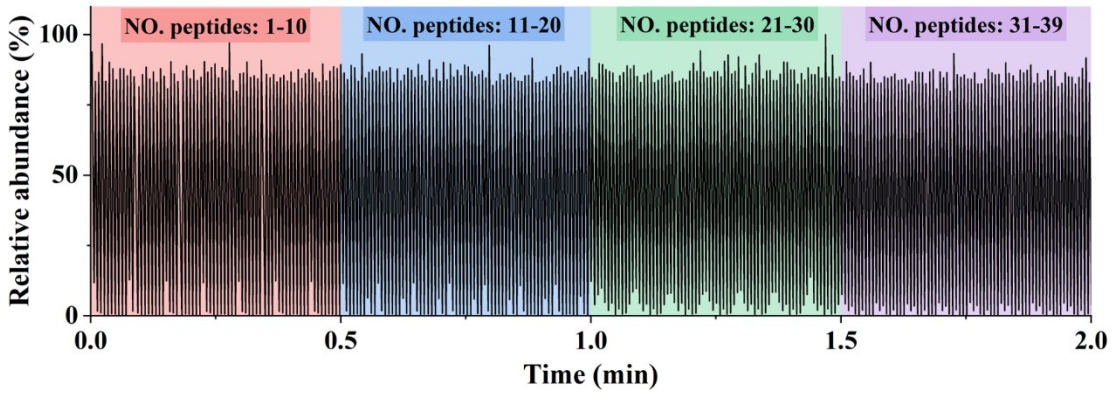

Fig. S11. The total ion chromatogram of abrin peptides obtained by nanoESI-HRMS analysis with the acquisition mode of full MS scan coupled with PRM.

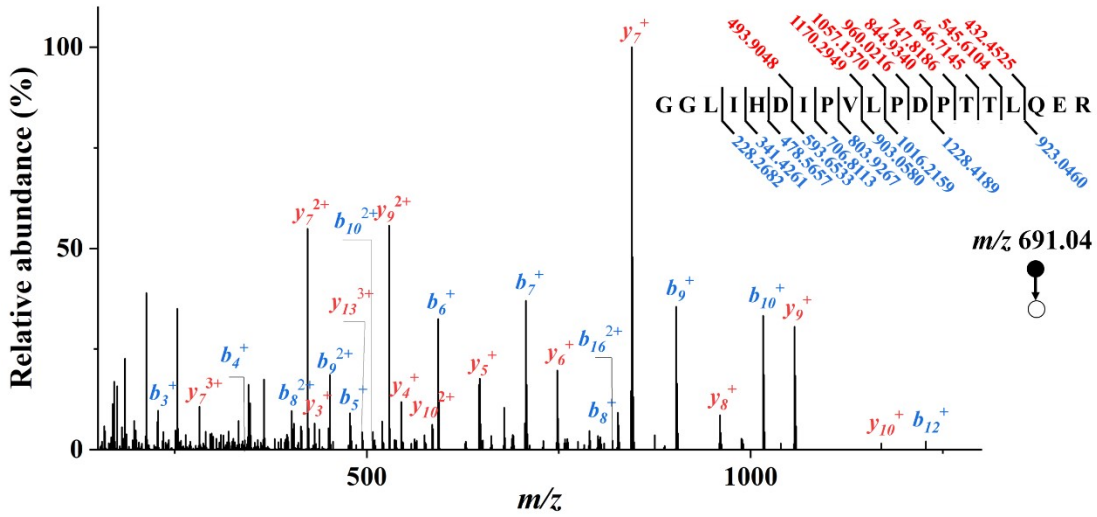

Fig. S12. MS/MS spectra of characteristic peptide (GGLIHDIPVLPDPTTLQER) hydrolyzed from abrin. The analysis was performed by nanoESI-MS/MS in positive mode.

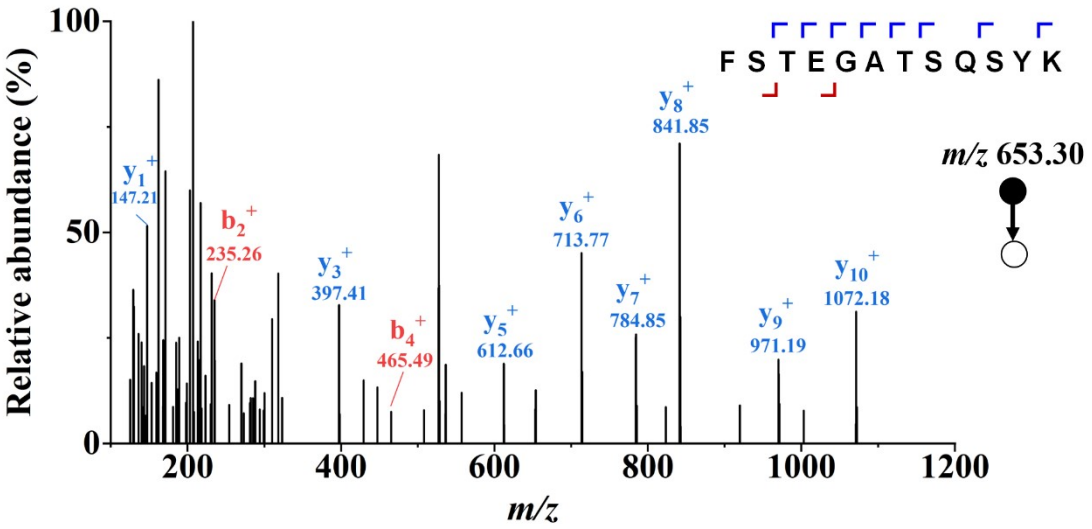

Fig. S13. MS/MS spectra of characteristic peptide (FSTEGATSQSYK) hydrolyzed from abrin. The analysis was performed by nanoESI-MS/MS in positive mode.

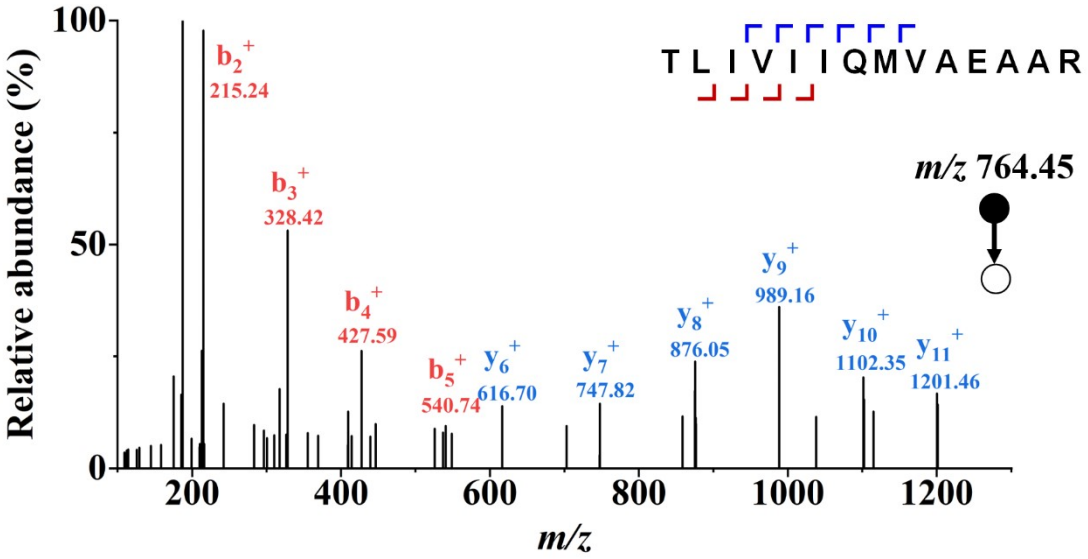

Fig. S14. MS/MS spectra of characteristic peptides (TLIVIIQMVAEAAR) hydrolyzed from abrin. The analysis was performed by nanoESI-MS/MS in positive mode.

References

1. B. F. Erlanger, N. Kokowsky and W. Cohen, *Archives of Biochemistry and Biophysics*, 1961, **95**, 271-278.
2. E.-M. Hansbauer, S. Worbs, H. Volland, S. Simon, C. Junot, F. Fenaille, B. G. Dorner and F. Becher, *Analytical Chemistry*, 2017, **89**, 11719-11727.
3. S. Worbs, B. Kampa, M. Skiba, E. M. Hansbauer, D. Stern, H. Volland, F. Becher, S. Simon, M. B. Dorner and B. G. Dorner, *Toxins (Basel)*, 2021, **13**.
4. L.-H. Liang, Y. Yang, S. Geng, X. Cheng, H.-L. Yu, C.-C. Liu and S.-L. Liu, *Journal*, 2021, **13**.
5. S. A. Fredriksson, E. Artursson, T. Bergstrom, A. Ostin, C. Nilsson and C. Astot, *Anal Chem*, 2015, **87**, 967-974.