Supplementary Materials

Experimental Methods

Synthesis of the SoPIL reagents. Unless otherwise noted, chemicals were purchased from Sigma-Aldrich (St. Louis, MO). 4-(4-formyl-3,5-dimethoxyphenoxy)butyric acid (**1**) (Novabiochem, CA) 268mg (1.0 mmol) was dissolved in DMF and MeOH (1:4 v/v) and mixed with aniline 250 mg (2.5 mmol) and HOAc 75 μL(1.25 mmol), followed by the addition of $NaBH_3CN$ 120 mg (2mmol). The reaction proceeded at room temperature for 2h (**1**). The solution was worked up and the product was extracted with EtOAc and purified by flash chromatography to give 260 mg (75%) NMR pure product 4-(3,5-Dimethoxy-4-phenylaminomethyl-phenoxy)-butyric acid (**2**). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.17 (t, *J* = 7.5 Hz, 2 H), 6.75 (d, *J* = 7.5 Hz, 2 H), 6.80 (t, *J* = 7.5 Hz, 1 H), 6.10 (s, 2 H), 4.27 (s, 2 H), 4.00 (t, *J* = 6.0 Hz, 2 H), 3.80 (s, 6 H), 2.58 (t, *J* = 7.2 Hz, 2 H), 2.14-2.04 (m, 2 H)

Bromoacetyl chloride (Alfa Aesar) 50 uL (0.6 mmol) in anhydrous 0.5 mL of THF and 0.6 mL of 1N NaOH were added dropwise to the THF solution of Compound (**2**) at 0°C. The reaction was continued at this temperature for 30 min. Then the reaction mixture was neutralized with 1N HCl at 0°C. The product was exacted with EtOAc and purified by flash chromatography to obtain 120 mg (75%) NMR pure product 4-(4-{[(2-Bromo-acetyl)-phenyl-amino]-methyl}-3,5-dimethoxy-phenoxy)-butyric acid (**3**). ^{1}H NMR ($CDCl_3$, 300

MHz): δ 7.26-7.21 (m, 3 H), 6.98-6.97 (m, 2 H), 5.92 (s, 2 H), 4.97 (s, 2 H), 3.96 (t, *J* = 6 Hz, 2 H), 3.61 (s, 2 H), 3.59 (s, 6 H), 2.58 (t, *J* = 7.5 Hz, 2 H), 2.11-2.05 (m, 2 H)

The heavy isotope form was synthesized similarly using aniline-$^{13}C_6$. 28 mg of PAMAM dendrimer Generation 4.0 (2 μmol) was dissolved in 10 mL of 200 mM MES (pH = 5.8) followed by the addition of 4-pentynoic acid 1.6 mg (16 μmol) in DMF 200 μL, N-hydroxy succinic anhydride (NHS) 20 mg and (1-[3-(dimethylamino)propyl]-3-ethylcarbiimide HCl (EDC) 200 mg. The solution was stirred at room temperature for 12 hours. After intensive dialysis in water, the solution was concentrated by ultrafiltration to 1 mL and split into halves for further reaction with compound (**3**) and the heavy form (**3**)-$^{13}C_6$, respectively. G4-PAMAM-(alkyne)$_6$ (1 μmol) prepared above was dissolved in 2 mL of 200 mM MES (pH = 5.8). Then 9.2 mg of compound (**3**)-$^{12}C_6$ or -$^{13}C_6$ in 2 mL of DMF was added to the above solution followed by the addition of 10 mg of NHS and 100 mg of EDC. The reaction was continued at room temperature for 12 hours in the dark. After intensive dialysis in water, the solution was concentrated by ultrafiltration to 600 μL which was directly used for capture test.

Synthesis of azide beads. Amino PEG4 azide (**4**) was prepared as reported in the literature (Schwabacher A. W., Lane, J. W., Leigh, K. M., Johnson, C. W. *J. Org. Chem.* **1998**, *63*, 1727-1729). Aminopropyl controlled pore glass beads (NH_2: ~400 μmol/g) (200 mg) was mixed with 80 mg of succinic anhydride in 400 μL

of DMF and 200 µL of pyridine. The reaction was allowed to proceed at room temperature overnight, and the beads were extensively washed. Amino PEG4 azide (**4**) (109 mg, 500 µmol) in 500 µL of DMF was added to the beads followed by the addition of HOBt (63 mg, 500 µmol) and 80 µL of DIPCI (500 µmol). The reaction was allowed to proceed at room temperature overnight, and the beads were extensively washed and dried in vacuum.

Synthesis of Cys-specific solid phase beads. 100 mg of aminopropyl controlled pore glass beads (NH_2: ~400 µmol/g) were washed with anhydrous DMF. 200 µmol each of 1-hydroxybenzotriazole (HOBt) (Nova Biochem, Laufelfingen, Switzerland), 4-(4-formyl-3,5-dimethoxyphenoxy)butyric acid (**1**) (Nova Biochem) and diisopropyl carbodiimide (DIPCI) were mixed for 30 min at room temperature, then this mixture was added to the beads for 90 min. The beads were then washed sequentially with DMF and dichloromethane and capped with a 2 ml mixture containing acetic anhydride:pyridine:dichloromethane (2:3:5) for 30 min. The beads were washed again with DMF and incubate with 20 mg of aniline (200 µmol) and 7.5 µL of HOAc (125 µmol) in 1 ml of DMF for 30 min, followed by the addition of $NaBH_3CN$ 12 mg (200 µmol). The reaction proceeded at room temperature for additional 2h (**1**). The beads were washed again with DMF and incubate with 200 µmol of iodoacetic anhydride and diisopropyl ethyl amine (DIEA) for 90 min. Beads were washed successively with DMF, water

and methanol, dried under reduced pressure and stored at room temperature in the dark.

Comparison of capturing cysteine-containing peptides and acid cleavage reactions using the SoPIL reagent and the direct solid phase approach. A peptide mixture consisting of 100 pmol of cysteine-containing Laminin B peptide (sequence: CDPGYIGSR) and 20 pmol non-cysteine containing angiotensin (sequence: DRVYIHPF) was used. Peptides were reduced with 5 mM tris(carboxyethyl) phosphine (TCEP) in 100 µl of 0.1 M Tris (pH 8.0) and 5 mM EDTA for 10 min at room temperature. 10 nmol of the SoPIL-$^{12}C_6$ reagent and 7 mg of the solid phase beads were prepared as described in the Supplementary Materials and were used to capture peptides in a volume of 100 µl under constant agitation. Aliquots of 1 µl of the supernatant were removed from the reaction mixture for MALDI-TOF/TOF analysis before the start of reactions and at different time points during the reaction. After 1 h of incubation, the reactions were quenched by the addition of 2 µl of 200 mM DTT for 5 min. For the reaction with the SoPIL reagent, 10 mg of azide beads was added in a total volume of 400 µL solution containing 2.5 mM of TCEP, 2.5 mM of $CuSO_4$, 0.25 mM of tris(triazolyl)amine for 1 hour. Both beads were washed successively by 2M NaCl, 80% acetonitrile and water. 100 µL of 90% TFA in water was added to the beads and incubate for 1 hour. The released peptides were collected by filtration and beads were washed with

80% acetonitrile twice. The elutions were combined and dried under vacuum for MS analysis.

Yield determination of capturing cysteine-containing peptides and acid cleavage reactions using the SoPIL reagent and the direct solid phase approach. A peptide mixture consisting of 100 pmol of cysteine-containing Laminin B peptide (sequence: CDPGYIGSR) and 20 pmol non-cysteine containing angiotensin (sequence: DRVYIHPF) was used. Peptides were reduced with 5 mM tris(carboxyethyl) phosphine (TCEP) in 100 µl of 0.1 M Tris (pH 8.0) and 5 mM EDTA for 10 min at room temperature. 10 nmol of the SoPIL-$^{12}C_6$ reagent and 7 mg of the solid phase beads-$^{13}C_6$ were prepared as described in the Supplementary Materials and were used to capture peptides in a volume of 100 µl under constant agitation. Aliquots of 1 µl of the supernatant were removed from the reaction mixture for MALDI-TOF/TOF analysis before the start of reactions and at different time points during the reaction. After 1 h of incubation, the reactions were quenched by the addition of 2 µl of 200 mM DTT for 5 min. For the reaction with the SoPIL reagent, 10 mg of azide beads was added in a total volume of 400 µL solution containing 2.5 mM of TCEP, 2.5 mM of $CuSO_4$, 0.25 mM of tris(triazolyl)amine for 1 hour. Both beads were combined and washed successively by 2M NaCl, 80% acetonitrile and water. 100 µL of 90% TFA in water was added to the beads and incubate for 1 hour. The released peptides were collected by filtration

and beads were washed with 80% acetonitrile twice. The elutions were combined and dried under vacuum. 20 pmol non-cysteine containing angiotensin II was added into the solution as standard. The products were analyzed by MALDI-TOF/TOF.

Isolation and analysis of snake venoms. Snake venoms A and B from *Crotalus scutulatus* (Mohave Rattlesnake) were obtained from Natural Toxins Research Center at Texas A&M-Kingsville. The venoms were collected from snake and lyophilized immediately. After receiving, samples (100 μg each) were immediately denatured in 8M urea, reduced with 5mM TCEP at 37 ºC for 30 min. Samples were diluted with 20 mM Tris pH 8.0, 1 mM EDTA. Each protein extract was digested by 5 μg trypsin overnight at 37 ºC. The resulting peptides from Snake venom A and B were labeled with the SoPIL light and heavy forms, respectively, combined, and processed as described above.

Fluorescence imaging of the delivery of SoPIL reagents in living cells. For fluorescence imaging experiments, the SoPIL reagent was functionalized with Fluorescein isothiocyanate (FITC). HeLa cells were grown to 50% confluency in DMEM/high glucose supplemented with 10% FBS, 100 μg/ml streptomycin sulfate, and 100 units/ml penicillin G in a 5% CO2 incubate at 37ºC. Cells were then treated with the SoPIL reagent in Cysteine-free media (Sigma) for

defined time period (3-5h) at 37ºC for the delivery of the synthesized dendrimers into living cells. Free extracellular SoPIL reagents were removed by washing with fresh medium and living cells were directly observed under fluorescence immmicroscopy.

Labeling and capturing Cys-containing proteins in living cells. HeLa cells (10^7) were grown to 80% confluent in DMEM/high glucose supplemented with 10% FBS, 100 μg/ml streptomycin sulfate, and 100 units/ml penicillin G in a 5% CO2 incubate at 37ºC. Cells were then treated with the SoPIL reagent in Cysteine-free media (Sigma) for 4h at 37ºC for the delivery of the synthesized dendrimers into living cells. Free extracellular SoPIL reagents were removed by washing with fresh medium. Cells were lysed and the whole cell extract were directly incubated with 10 mg azide beads in a total volume of 400 μL solution containing 2.5 mM of TCEP, 2.5 mM of $CuSO_4$, 0.25 mM of tris(triazolyl)amine for 1 hour. The beads were incubated with 8M urea at 37ºC for 30 min to denature the proteins. The beads were extensively washed with lysis buffer and 100 mM Tris buffer and directly incubated with 1 μg of trypsin in 100 μl of 100 mM Tris (pH8.0) for overnight digestion at 37C. The beads were washed sequentially with 2.0 M sodium chloride, 80% acetonitrile and water. Modified Cys-containing peptides were recovered with acid cleavage and dried under vacuum for MS analysis

Peptide and protein identification and quantification by μLC-MS/MS. The Agilent 1100 HPLC system was coupled online with LTQ linear ion trap mass spectrometer (Thermoelectron, San Jose, CA). Both MS and MS/MS spectra were acquired with the instrument operating in the data-dependent mode of one MS scan followed by ten MS/MS scans. All MS/MS spectra were searched against protein databases using the SEQUEST™ algorithm on a Lynx cluster or the Sorcerer™ IDA server (SageN, Inc, San Jose, CA). The validation of protein identification and quantification were performed with Trans-Peptide Pipeline (TPP) software developed by Institute for Systems Biology in Seattle.

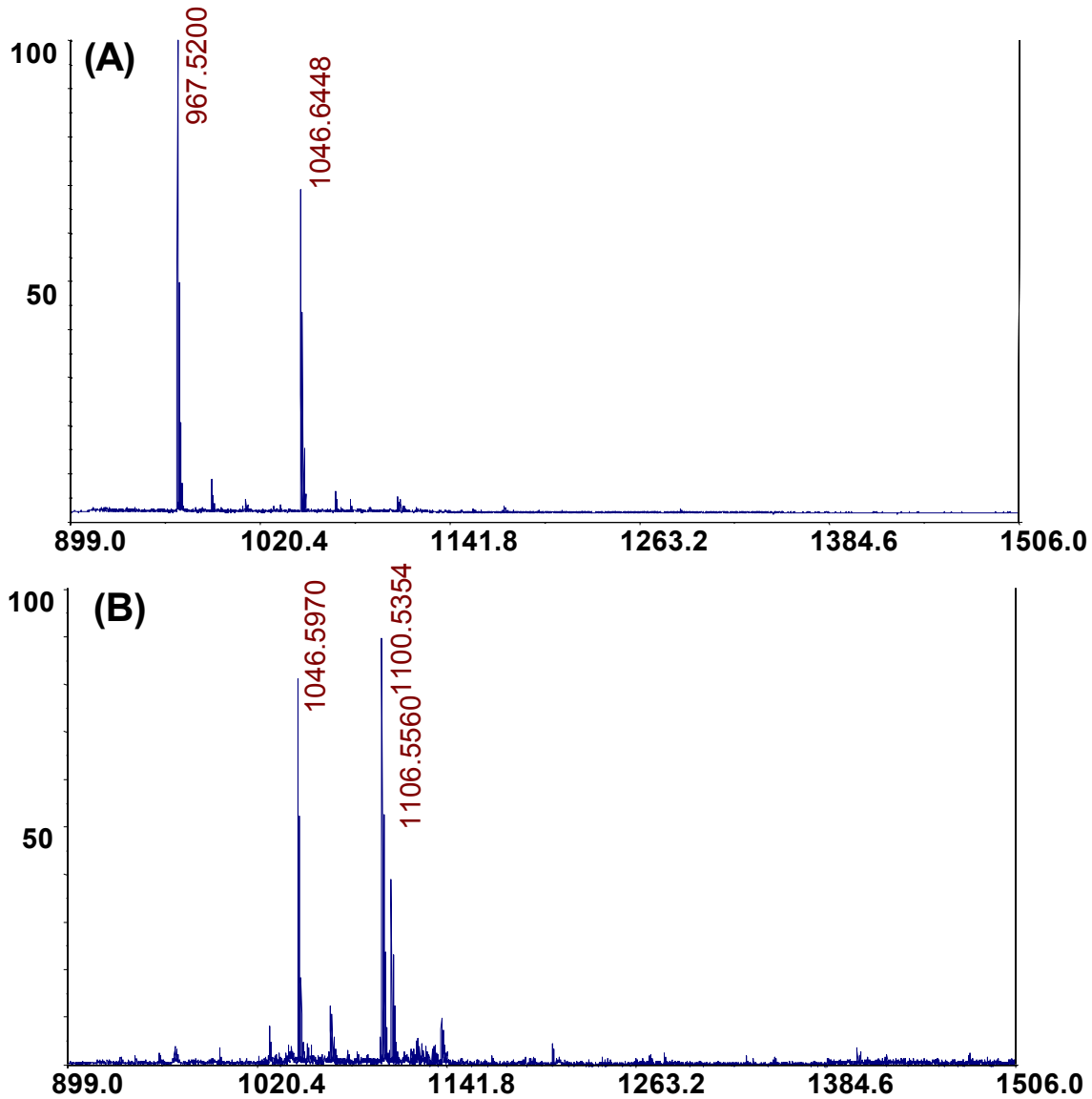

Fig. S1 Comparison between the solid phase method and SoPIL approach. (A) Prior to the reaction. (B) Acid cleaved products from the solid phase (with heavy isotope, m/z 1106.6) and SoPIL methods (with light isotope, m/z 1100. 5). The m/z 1046 was added as standard. The m/z 967 ion was converted to m/z 1100 in the SoPIL method and to m/z 1106 in the solid phase method. See Supplementary Information method section for experimental details.

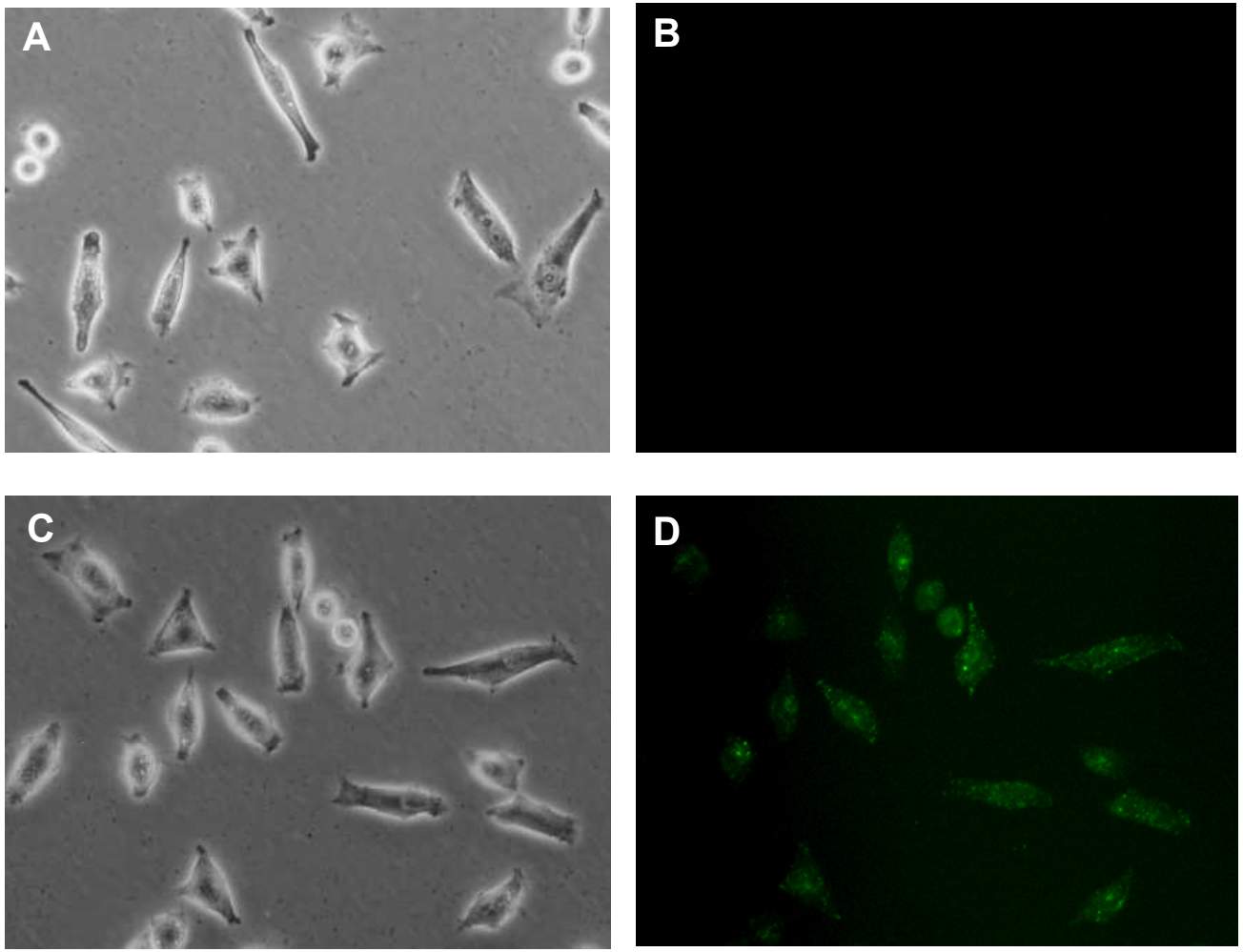

Fig. S2 Phase contrast and fluorescence microscopic images of HeLa cells with SoPIL reagents. HeLa cells without any treatment as the control phase contrast image (A) and fluorescence image (B); HeLa cells treated with 5 mM SoPIL for 4h phase contrast image (C) and fluorescence image (D).

Table S1

probability	Sequest xcorr	peptide sequence	protein	asap ratio	asap error
0.9992	4.157	K.YICDNQDTISSK.L	ALBU_BOVIN	0.93	0.12
0.9899	3.524	K.YIC*DNQDTISSK.L	ALBU_BOVIN	0.93	0.12
0.9843	3.032	K.TCVADESHAGCEK.S	ALBU_BOVIN	0.85	0.11
0.9833	2.557	K.TC*VADESHAGC*EK.S	ALBU_BOVIN	0.85	0.11
0.9896	3.596	R.RPC*FSALTPDETYVPK.A	ALBU_BOVIN	1.26	0.15
0.9989	3.611	R.RPCFSALTPDETYVPK.A	ALBU_BOVIN	1.26	0.16
0.9996	3.743	R.RPC*FSALTPDETYVPK.A	ALBU_BOVIN	1.25	0.17
0.9991	3.845	K.EYEATLEECCAK.D	ALBU_BOVIN	1	0.17
0.9985	3.139	K.EYEATLEEC*C*AK.D	ALBU_BOVIN	0.98	0.16
0.9991	4.48	K.YNGVFQEC*C*QAEDK.G	ALBU_BOVIN	1.08	0.17
0.9937	4.641	K.YNGVFQECCQAEDK.G	ALBU_BOVIN	1.08	0.17
0.9588	2.755	L.SFNPTQLEEQC*HI.-	LACB_BOVIN	0.46	0.08
0.9561	3.648	R.LSFNPTQLEEQC*HI.-	LACB_BOVIN	0.58	0.16
0.9935	4.437	K.FLDDDLTDDIMCVK.K	LALBA_BOVIN	1.88	0.29
0.8462	3.223	H.SSNIC*NISC*DK.F	LALBA_BOVIN	2.21	0.25
0.9968	2.349	R.C*ELAAAMK.R	LYSC_CHICK	0.84	0.12
0.9974	2.812	R.CELAAAMK.R	LYSC_CHICK	0.84	0.12
0.978	4.756	C.SALLSSDITASVNC*AK.K	LYSC_CHICK	0.91	0.17
0.9339	4.879	C.SALLSSDITASVNCAK.K	LYSC_CHICK	0.91	0.17
0.9868	5.217	R.NLCNIPCSALLSSDITASVNCAK.K	LYSC_CHICK	0.94	0.15
0.9756	4.883	R.NLC*NIPC*SALLSSDITASVNC*AK.K	LYSC_CHICK	0.94	0.15

C and C* refer to cysteine residues labeled with light or heavy SoPIL reagents, respectively.
M#: oxidized methine residue.

Expected ratio	Protein average ratio
1	1.04
1	
1	
1	
1	
1	
1	
1	
1	
1	
1	
0.5	0.52
0.5	
2	2.04
2	
1	0.9
1	
1	
1	
1	
1	

Table S3. Example of two sname proteins that were cleavged extensively by endogenous proteases. Note that the peptides were identified by searching against the database with at least one tryptic site.

>gi|231997|sp|P30431|DISJ_BOTJA Putative venom metalloproteinase jararhagin precursor (HF2-proteinase) [Contains: Disintegrin]
ATRPKGAVQP KYEDAMQYEF KVNGEPVVLH LEKNKGLFSK DYSEIHYSPD GREITTYPPV
EDHCYYHGRI ENDADSTASI SACNGLKGYF KLQRETYFIE PLKLPDSEAH AVFKYENVEK
EDEAPKMCGV TQNWKSYEPI KKASQLAFTA EQQRYDPYKY IEFFVVVDQG TVTKNNGDLD
KIKARMYELA NIVNEIFRYL YMHVALVGLE IWSNGDKITV KPDVDYTLNS FAEWRKTDLL
TRKKHDNAQL LTAIDFNGPT IGYAYIGSMC HPKRSVGIVQ DYSPINLVVA VIMAHEMGHN
LGIHHDTGSC SCGDYPCIMG PTISNEPSKF FSNCSYIQCW DFIMNHNPEC IINEPLGTDI
ISPPVCGNEL LEVGEECDCG TPENCQNECC DAATCKLKSG SQCGHGDCCE QCKFSKSGTE
CRASMSECDP AEHCTGQSSE CPADVFHKNG QPCLDNYGYC YNGNCPIMYH QCYALFGADV
YEAEDSCFKD NQKGNYYGYC RKENGKKIPC APEDVKCGRL YCKDNSPGQN NPCKMFYSND
DEHKGMVLPG TKCADGKVCS NGHCVDVATA Y

Observed Peptides

Position	Mass	Peptide
60-69	1278.36	VEDHCYYHGR
381-396	1729.89	TPENCQNECCDAATCK
385-396	1288.45	CQNECCDAATCK
397-413	1783.00	LKSGSQCGHGDCCEQCK
399-413	1541.66	SGSQCGHGDCCEQCK
401-413	1397.53	SQCGHGDCCEQCK
404-413	1079.19	GHGDCCEQCK
405-413	1022.13	HGDCCEQCK
422-448	2923.17	RASMSECDPAEHCTGQSSECPADVFHK
423-448	2766.98	ASMSECDPAEHCTGQSSECPADVFHK
423-447	2638.81	ASMSECDPAEHCTGQSSECPADVFH
424-448	2695.90	SMSECDPAEHCTGQSSECPADVFHK
426-448	2477.63	SECDPAEHCTGQSSECPADVFHK
429-448	2158.30	DPAEHCTGQSSECPADVFHK
430-448	2043.21	PAEHCTGQSSECPADVFHK
431-448	1946.10	AEHCTGQSSECPADVFHK
432-448	1875.02	EHCTGQSSECPADVFHK
433-448	1745.90	HCTGQSSECPADVFHK
436-448	1404.52	GQSSECPADVFHK
449-459	1243.31	NGQPCLDNYGY
449-464	1794.89	NGQPCLDNYGYCYNGN
449-461	1509.63	NGQPCLDNYGYCY
493-501	1123.25	KGNYYGYCR
494-501	995.08	GNYYGYCR
508-516	971.14	IPCAPEDVK
524-534	1173.22	DNSPGQNNPCK

558-570 1275.42 VCSNGHCVDVATA

558-571 1438.59 VCSNGHCVDVATAY

>gi|13959638|sp|Q9DF66|VSP3_TRIJE Venom serine proteinase 3 precursor (SP3)
MVLIRVLANL LILQLSYAQK SSELIIGGHP CNINEHRSLV VLFNSSGLLC SGTLINKEWV
LTAAHCDSNN FQLLFGVHSK KVLNEDEQTR DPKEKFICPN KKKDDEKDKD IMLIRLDSSV
SNSEHIAPLS LPSSPPSVGS ACRVMGWGKT IPTKDTYPDV PHCANINILD HAVCRAAYSN
LLEKSKTLCA GILQGGKDTC QFDSGGPLIC NGQVQGIVSW GGHPCGQPHA LGVYTNVFNY
TDWIQSIIAG NTDATCPP

Observed Peptides

Position	Mass	Peptide
25-37	1459.65	IIGGHPCNINEHR
27-37	1233.33	GGHPCNINEHR
29-37	1119.22	HPCNINEHR
125-143	1876.16	HIAPLSLPSSPPSVGSACR
129-143	1457.66	LSLPSSPPSVGSACR
130-143	1344.51	SLPSSPPSVGSACR
131-143	1257.43	LPSSPPSVGSACR
155-175	2366.65	DTYPDVPHCANINILDHAVCR
158-175	1987.28	PDVPHCANINILDHAVCR
160-175	1775.08	VPHCANINILDHAVCR
161-175	1675.94	PHCANINILDHAVCR
162-175	1578.83	HCANINILDHAVCR
163-175	1441.69	CANINILDHAVCR
165-175	1267.47	NINILDHAVCR
166-175	1153.36	INILDHAVCR