

## Supporting information

Snake venom characteristic peptides: novel fingerprints for species identification by sheathless capillary electrophoresis - electrospray ionization - mass spectrometry

Ying Liu,<sup>†a</sup> Xiao-Hui Zhang,<sup>†b</sup> Yue Yu,<sup>a</sup> Hong-Xu Chen,<sup>\*c</sup> Ying-Lin Zhou <sup>\*a</sup> and Xin-Xiang Zhang <sup>\*a</sup>

*a. Beijing National Laboratory for Molecular Sciences (BNLMS), Key Laboratory of Bioorganic Chemistry and Molecular Engineering of Ministry of Education, College of Chemistry and Molecular Engineering, Peking University, Beijing 100871, China. E-mail: zhouyl@pku.edu.cn; zxx@pku.edu.cn.*

*b. State Key Laboratory of Natural and Biomimetic Drugs, Peking University, Beijing 100191, China.*

*c. SCIEX CHINA, Beijing 100015, China. E-mail: chx229@126.com.*

† These authors contributed equally to this work.

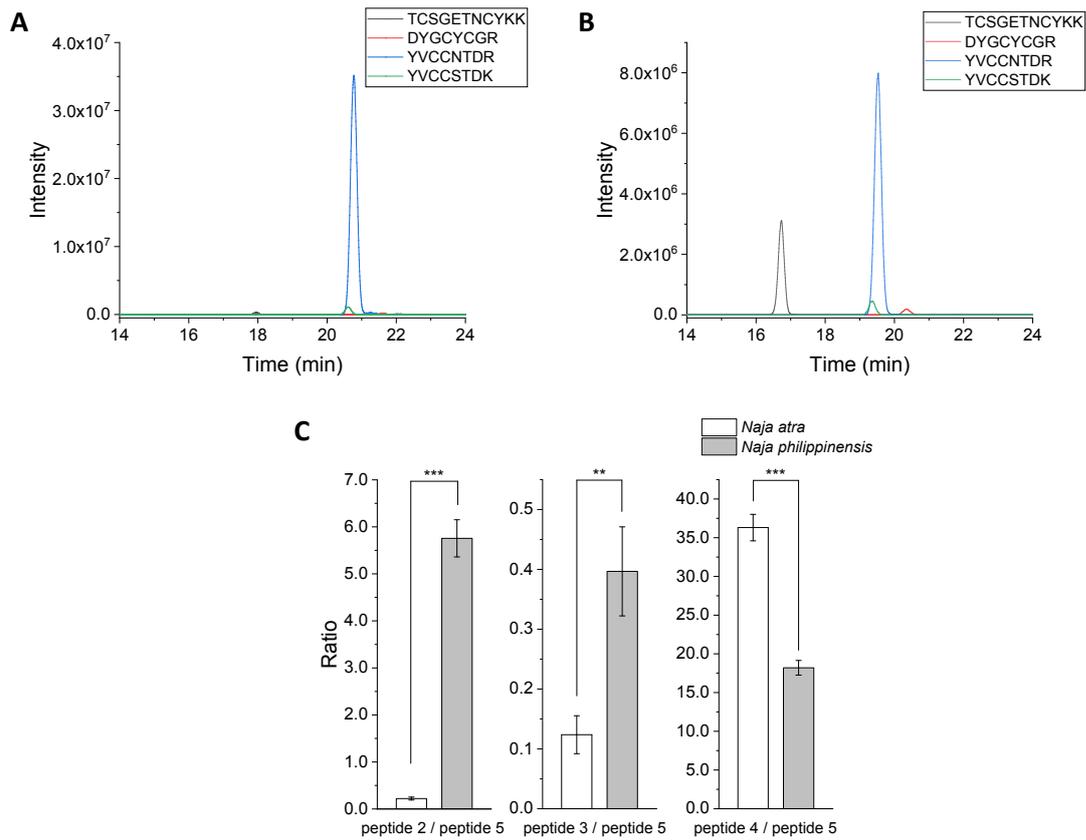


Fig. S1 Multiple extracted ion electropherograms for the characteristic peptides existed in both *Naja atra* and *Naja philippinensis*. (A) 10  $\mu\text{g}/\text{mL}$  of *Naja atra* venom peptides. (B) 10  $\mu\text{g}/\text{mL}$  of *Naja philippinensis* venom peptides. (C) Peak area ratios of the four characteristic peptides existed in both *Naja atra* and *Naja philippinensis*. Peptide 2: TCSGETNCYKK; peptide 3: DYGCYCGR; peptide 4: YVCCNTDR; peptide 5: YVCCSTDK. The two snake venoms were prepared in same three ways: 10  $\mu\text{g}/\text{mL}$  of venom peptides; 1  $\mu\text{g}/\text{mL}$  of venom peptides; 1% venom peptides in digested beta-gal. For statistical analysis, Bonferroni's multiple comparison test was used. \*  $P < 0.05$ ; \*\*  $P < 0.01$ ; \*\*\*  $P < 0.001$ .

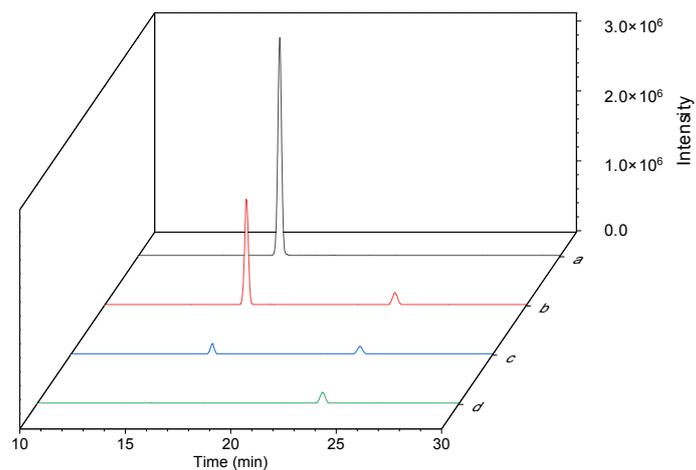


Fig. S2 Extracted ion electropherograms ( $m/z = 674.2873$ ) for the mixture of *Naja philippinensis* venom digested peptides and digested beta-gal peptides. (a)  $10 \mu\text{g/mL}$  of *Naja philippinensis* venom peptides; (b) 1:1 of  $10 \mu\text{g/mL}$  of *Naja philippinensis* venom peptides and  $1 \text{ mg/mL}$  digested beta-gal peptides; (c) 1:1 of  $1 \mu\text{g/mL}$  of *Naja philippinensis* venom peptides and  $1 \text{ mg/mL}$  digested beta-gal peptides; (d)  $0.5 \text{ mg/mL}$  digested beta-gal peptides.

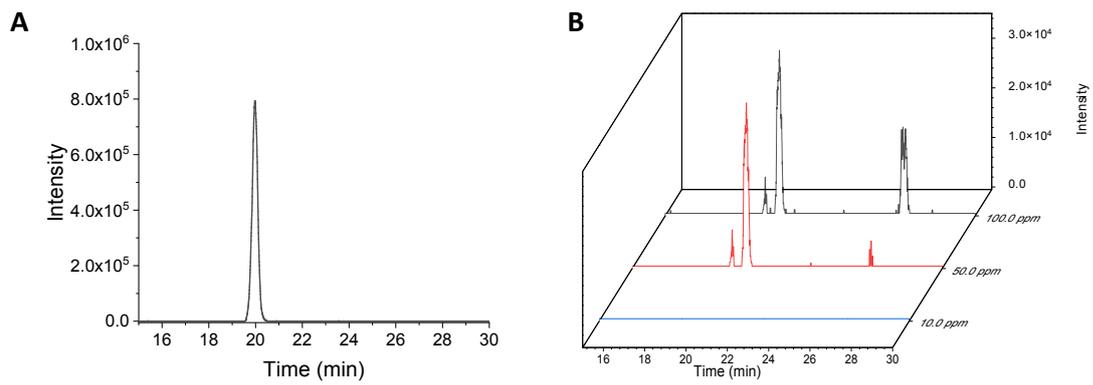


Fig. S3 Extracted ion electropherograms of *Bungarus multicinctus* characteristic peptide ECAATCPPSK. (A) 10  $\mu\text{g/mL}$  of *Bungarus multicinctus* venom peptides, migration time: 19.98 min, mass tolerance 10.0 ppm. (B) 10  $\mu\text{g/mL}$  of *Deinagkistrodon acutus* venom peptides, migration time of the 3 peaks: 19.84, 20.53, 26.50 min; (Black) mass tolerance 100.0 ppm; (Red) mass tolerance 50.0 ppm; (Blue) mass tolerance 10.0 ppm.

**Table S1** Characteristic sequence information of different species of snake venom.

Species of snakes	Characteristic peptides	m/z	MW	Migration times (min)	Maching proteins and species	
					Species	Numbers of proteins
<i>Naja atra</i> (Jiangxi, China)	YVCCSTDK	516.7104	1031.4053	20.60	<i>Naja atra</i>	3
					<i>Naja naja</i>	1
					<i>Naja kaouthia</i>	1
					<i>Naja nivea</i>	1
					<i>Naja annulifera</i>	1
	YVCCNTDR	544.2188	1086.4222	20.77	<i>Naja atra</i>	20
					<i>Naja sputatrix</i>	13
					<i>Naja kaouthia</i>	7
					<i>Naja naja</i>	4
					<i>Naja oxiana</i>	2
					<i>Naja annulifera</i>	2
					<i>Naja nivea</i>	1
	<i>Naja melanoleuca</i>	1				
	MFMVATPK	462.7374	923.4609	20.24	<i>Naja atra</i>	7
					<i>Naja sputatrix</i>	2
					<i>Naja kaouthia</i>	1
	DMVECCSTDR	636.7363	1271.4580	22.66	<i>Naja sputatrix</i>	4
<i>Naja atra</i>					2	
<i>Naja kaouthia</i>					2	
<i>Naja annulifera</i>					1	
DYGCYCGR	525.6920	1049.3695	21.62	<i>Naja sputatrix</i>	4	
				<i>Naja mossambica</i>	3	
				<i>Naja nigricollis</i>	3	

					<i>Naja sagittifera</i>	3
					<i>Naja atra</i>	2
					<i>Naja kaouthia</i>	2
					<i>Naja pallida</i>	1
					<i>Naja naja</i>	1
					<i>Micrurus altirostris</i>	1
<i>Bungarus multicinctus</i>	ICHFDTCR	370.1599	1107.4590	15.68	<i>Bungarus multicinctus</i>	2
	DCDKPPDTR	368.4970	1102.4691	16.96	<i>Bungarus candidus</i>	4
					<i>Bungarus multicinctus</i>	3
					<i>Bungarus fasciatus</i>	2
	HPDCDKPPDTR	446.5351	1336.5830	14.71	<i>Bungarus candidus</i>	4
					<i>Bungarus multicinctus</i>	3
					<i>Bungarus fasciatus</i>	2
	ECAATCPPSK	560.7421	1119.4697	19.98	<i>Bungarus multicinctus</i>	3
TCPSGQLLCLK	638.8227	1275.6309	20.24	<i>Bungarus multicinctus</i>	1	
				<i>Bungarus candidus</i>	1	
<i>Daboia russellii</i>	HIAPVSLPSR	538.8145	1075.6138	18.55	<i>Daboia siamensis</i>	2
					<i>Gloydius ussuriensis</i>	2
					<i>Trimeresurus stejnegeri</i>	2
					<i>Protobothrops jerdonii</i>	2
					<i>Gloydius shedaoensis</i>	1
					<i>Trimeresurus gramineus</i>	1
					<i>Protobothrops flavoviridis</i>	1
					<i>Deinagkistrodon acutus</i>	1
					<i>Vipera berus nikolskii</i>	1
	LNKPVTYST	511.7800	1021.5444	22.05	<i>Daboia siamensis</i>	1
HDRPTFCN	523.7275	1045.4404	17.97	<i>Daboia siamensis</i>	7	

					<i>Daboia russelii</i>	2
	LVEYSYSYR	590.2877	1178.5609	22.67	<i>Daboia siamensis</i>	2
					<i>Daboia russelii</i>	1
	HTCGASGNVGRPR	489.2407	1464.7002	16.98	<i>Daboia siamensis</i>	1
					<i>Daboia russelii</i>	1
<i>Gloydius brevicaudus</i>	DNLNTYNDKK	612.7972	1223.5782	17.50	<i>Gloydius halys</i>	3
					<i>Gloydius ussuriensis</i>	1
					<i>Muriicola sp. MMS17-SY002</i>	1
					<i>Clostridium sp. KLE 1755</i>	1
	YDSNLDTIR	548.7676	1095.5197	20.78	<i>Gloydius brevicaudus</i>	2
					<i>Gloydius halys</i>	1
	CGSPGNPCCDAATCK	827.8075	1653.6003	22.58	<i>Gloydius brevicaudus</i>	3
					<i>Xenopus laevis</i>	3
					<i>Gloydius halys</i>	2
					<i>Crotalus viridis viridis</i>	2
					<i>Crotalus atrox</i>	2
					<i>Gloydius ussuriensis</i>	1
					<i>Gloydius blomhoffii</i>	1
					<i>Crotalus scutulatus scutulatus</i>	1
					<i>Crotalus ruber ruber</i>	1
<i>Austrofundulus limnaeus</i>					1	
<i>Collichthys lucidus</i>	1					
<i>Astyanax mexicanus</i>	1					
EGTICQEAK	518.2423	1034.4702	20.34	<i>Gloydius brevicaudus</i>	2	
EQATIFPCAQK	646.8188	1291.6230	21.59	<i>Gloydius brevicaudus</i>	2	
<i>Naja philippinensis</i>	LECHNQSSQAPTTK	576.5770	1727.7897	18.32	<i>Naja sputatrix</i>	3
					<i>Naja philippinensis</i>	1

					<i>Naja kaouthia</i>	1
					<i>Naja samarensis</i>	1
	TCSGETNCYKK	674.2873	1346.5594	16.74	<i>Naja kaouthia</i>	2
					<i>Naja philippinensis</i>	1
					<i>Naja atra</i>	1
					<i>Naja samarensis</i>	1
					<i>Naja sputatrix</i>	1
					<i>Naja oxiana</i>	1
	DYGCYCGR	525.6920	1049.3695	20.35	<i>Naja sputatrix</i>	4
					<i>Naja nigricollis</i>	3
					<i>Naja sagittifera</i>	3
					<i>Naja mossambica</i>	3
					<i>Naja kaouthia</i>	2
					<i>Naja atra</i>	2
					<i>Naja pallida</i>	1
					<i>Naja naja</i>	1
					<i>Micrurus altirostris</i>	1
	YVCCNTDR	544.2188	1086.4222	19.54	<i>Naja atra</i>	21
					<i>Naja sputatrix</i>	13
					<i>Naja kaouthia</i>	7
					<i>Naja naja</i>	4
					<i>Naja oxiana</i>	2
					<i>Naja annulifera</i>	2
					<i>Naja nivea</i>	1
					<i>Naja melanoleuca</i>	1
	YVCCSTDK	516.7104	1031.4053	19.36	<i>Naja atra</i>	3
					<i>Naja kaouthia</i>	1

					<i>Naja naja</i>	1	
					<i>Naja nivea</i>	1	
					<i>Naja annulifera</i>	1	
<i>Deinagkistrodon acutus</i>	YVETVFFVVDK	599.8218	1197.6290	20.93	<i>Deinagkistrodon acutus</i>	1	
	HDEGSCSCGSG	576.6884	1151.3623	20.44	<i>Deinagkistrodon acutus</i>	6	
	FKEEGTICR	570.2798	1138.5450	16.27	<i>Deinagkistrodon acutus</i>	2	
					<i>Oryctolagus cuniculus</i>	1	
	YNGDLDKIK		524.7695	1047.5244	22.77	<i>Deinagkistrodon acutus</i>	2
						<i>Agkistrodon bilineatus</i>	1
						<i>Protobothrops mucrosquamatus</i>	1
IMSPVINSEVIK	665.3770	1328.7395	21.24	<i>Deinagkistrodon acutus</i>	6		
<i>Trimeresurus stejnegeri</i>	DNKDTYDNK	556.7463	1111.4780	18.09	<i>Trimeresurus gramineus</i>	4	
					<i>Trimeresurus stejnegeri</i>	2	
					<i>Bothrops moojeni</i>	1	
					<i>Bothrops erythromelas</i>	1	
					<i>Bothrops pictus</i>	1	
					<i>Bothrops alternatus</i>	1	
					<i>Candidatus Magnetomorum sp. HK-1 [1509431]</i>	1	
	DNKDTYDWK	592.7638	1183.5131	18.31	<i>Trimeresurus stejnegeri</i>	2	
	DNLDTYKK	498.7533	995.4921	17.28	<i>Trimeresurus stejnegeri</i>	2	
					<i>Anaeromyces robustus</i>	1	
					<i>Nitratiruptor sp. (strain SB115-2) [387092]</i>	1	
NIFHPTSSCVK	645.3187	1288.6229	17.59	<i>Trimeresurus stejnegeri</i>	1		
MTNKEPILSYSK	476.2463	1425.7170	18.55	<i>Trimeresurus stejnegeri</i>	1		
<i>Gloydus ussuriensis</i>	DNLNTYNDKK	612.7968	1223.5782	18.49	<i>Gloydus halys</i>	2	

					<i>Gloydius ussuriensis</i>	1
					<i>Muriicola sp. MMS17-SY002</i>	1
					<i>Clostridium sp. KLE 1755</i>	1
	WDDYTYSWK	632.2703	1262.5244	24.55	<i>Gloydius halys</i>	3
					<i>Gloydius ussuriensis</i>	1
					<i>Gloydius blomhoffii</i>	1
					<i>Thermoclostridium stercorarium</i> (strain ATCC 35414 / DSM 8532 / NCIMB 11754) [1121335]	1
	MTGKEPVVSYA	591.2972	1180.5798	23.92	<i>Gloydius halys</i>	4
					<i>Gloydius ussuriensis</i>	1
					<i>Sphingobium algorifonticola</i>	1
	IYMAYPDIFCSSK	797.8679	1593.7207	26.88	<i>Gloydius ussuriensis</i>	1
					<i>Fonticella tunisiensis</i>	1
					<i>Clostridium intestinale URNW</i>	1
	KMTGKEPVVSYA	655.3432	1308.6718	18.18	<i>Gloydius halys</i>	4
					<i>Gloydius ussuriensis</i>	1
					<i>Sphingobium algorifonticola</i>	1