

Supplementary information

Nanobody-based recombinant antivenom for cobra, mamba, and rinkhals bites

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Supplementary Table 1. Snake species abbreviations, fraction names, toxin FASTA sequences, and content of the venom fractions used for phage display selection and screening of V_HHs. Key toxins used for labeling each fraction are highlighted in bold. The toxins used for labeling of the fractions were based on the clinically most relevant one.

To facilitate reading, this table is included as an Excel file.

Supplementary Table 2. Immunization scheme for each camelid animal, including the time points for phage display library construction.

Day/week	Total venom dose (mg)	Dose per venom (mg)	Library generation
0 d	0.27	0.015	No
14 d	0.54	0.03	No
28 d	0.72	0.04	No
42 d	0.99	0.06	No
46 d	-	-	Yes (Library A)
49 d	-	-	
56 d	1.26	0.07	No
70 d	1.44	0.08	No
84 d	1.80	0.10	No
98 d	2.16	0.12	No
102 d	-	-	Yes (Library B)
105 d	-	-	
52 w	4.5	0.25	No
54 w	4.5	0.25	No
60 w	4.5	0.25	No
60 w + 5 d			Yes (Library C)
60 w + 8 d			

Supplementary Table 3. Overview of the phage display campaigns. Toxin (sub)family, library, venom fraction or toxin, and the target toxin concentrations have been included for each round of panning. The labels for phage pool outputs selected for screening and the V_HHs characterized from each selection are shown in the last 2 columns.

Toxin (sub)family	Round 1			Round 2		Round 3		Selection number	Characterized V _H Hs
	Library	Target	Concentration (nM)	Target	Concentration (nM)	Target	Concentration (nM)		
CTx	A + B	CTx-10	50	CTx-7	100	CTx-10	50	TPL0870	V _H H1, V _H H2, V _H H3
	C	CTx-12	100	CTx-11	100	-	-	TPL1054	V _H H4
sNTx	C	sNTx-1	100	sNTx-1	100	sNTx-1	10	TPL0998	V _H H5, V _H H6
						sNTx-3	10	TPL1000	V _H H7
						sNTx-6	10	TPL1006	V _H H8
INTx	A + B	INTx-1	50	INTx-1	50	INTx-1	50	TPL0830	V _H H12
	A + B	INTx-7	100	INTx-7	50	INTx-3	5	TPL0833	V _H H10, V _H H11
						INTx-3	50	TPL0877	V _H H9
AgTx	C	AgTx-2	100	AgTx-2	100	AgTx-2	1	TPL1033	V _H H13, V _H H14
Og XI	C	Og XI-1	100	Og XI-1	100	Og XI-1	10	TPL1019	V _H H16
	C	Og XI-2	100	Og XI-2	100	Og XI-2	10	TPL1039	V _H H15
PLA ₂	B	PLA ₂ -3	50	PLA ₂ -3	50	-	-	TPL0599	V _H H19, V _H H20
					5	-	-	TPL0615	V _H H21
KUN	C	KUN-1	100	KUN-1	100	KUN-1	10	TPL1011	V _H H18
						KUN-2	10	TPL1013	V _H H17, V _H H19

Supplementary Table 4. Sequence of CDR3 (hit clones) or whole sequence (lead clones) for included V_HHs.

V _H H	Target	Amino acid sequence
V _H H1 a-CTx	CTx	QVQLQESGGGLVQA GGSRLRLSCAASGRTFSSYAMAWFRQAPGKEREFVASISWNGDSTYYADSVKGR FTISGDNAKNTWYLQMKSLKPEDTAVYYCNTEDEGSGTYIEWGQGTQVTVSSAAADYKDHDGDYK DHDIDYKDDDDKGAAHHHHHH
V _H H4 a-CTx	CTx	QVQLQESGGGLVQA GGSRLRLSCAAPGFTFDNYAIGWFRQAPGKEREA VSMIDVNDGDTYYADSVKGR FATSIDNARITAYLQMNSLKPEDTAVYYCAAGEQGGIETEDDYGMDDYKGTQVTVSSAAADYKDH DGDYKDHDIDYKDDDDKGAAHHHHHH
V _H H5 a-sNTx	sNTx	QVQLQESGGGLVRPGGSLRLSCA VSGVSEIFANYVVGWFRQQDSGTGRDLVAQSSSDDEYNHVSGSVK GRFTISRDNANKNTVSLQMDNLKPEDTAIYICAAAGIPGDNYIYW GQGTQVTVSSAAADYKDHDGDYK DHDIDYKDDDDKGAAHHHHHH
V _H H9 a-INTx	INTx	QVQLQESGGGLVQPGGSLRLSCTASGSIFSTVNTMGWYRQAPGKQRELVAAITGGGSTNYADSVKGRF TISRDNVENTVHLRMNALKPEDTAVYYCNFQEYCSA YGCYELPHDYWGQGTQVTVSSAAADYKDHD GDYKDHDIDYKDDDDKGAAHHHHHH
V _H H13 a-AgTx	AgTx	QVQLQESGGGLVQA GGSRLRLSCAASGLDFSTMPLTVGWFRQAPGKEREFVAAISWRGDSWNYEDGM EGRFTVSRDNAKNTVYLQMSLHTEDTGVYYCAGDPTPYGTSSGGFSVWGQGTQVTVSSAAADYKDH DGDYKDHDIDYKDDDDKGAAHHHHHH
V _H H15 a-Og XI	Og XI	QVQLQESGGGA VPA GGSRLTLSCAASGRTLSDYTMGWFRQAPGKEREIFGAISWNDGYTYDDSVKGR FTISRDNANKNTMYLQMNSLKPEDTAVYYCAAQAPEMVRSGNFGSWGPGTQVTVSSAAADYKDHDG DYKDHDIDYKDDDDKGAAHHHHHH
V _H H17 a-KUN	KUN	QVQLQESGGGLVQPGGSLRLSCITSGSNFDDLSVGWFRQAPGKEREGVSCFSSSDGSTYYVDSVKGRFTM SRDNAKNTVYLQMNNLKPEDTAVYYCTADGFPYPSETMCSIPGGPDSVAWGQGTQVTVSSAAADYK DHDGDYKDHDIDYKDDDDKGAAHHHHHH
V _H H20 a-PLA ₂	PLA ₂	QVQLQESGGGLAQAGGSRLRLSCAASGLAFSNYSMGWARQAPGKEREFVAAISWSHQPTYADSVKG RFTISRDNANKNTLYLQMNSLKPEDTAVYYCAADSRPNSYRLGGPGNYWGQGTQVTVSSAAADYKDH DGDYKDHDIDYKDDDDKGAAHHHHHH

V _H H	Target	Amino acid sequence CDR3
V _H H2 a-CTx	CTx	AANEPEDCSGSACDGS
V _H H3 a-CTx	CTx	SPDYGLVDTASCEYGY
V _H H6 a-sNTx	sNTx	AAALGPGDNYIYY
V _H H7 a-sNTx	sNTx	AAAPGDQYTN
V _H H8 a-sNTx	sNTx	AAALGPGDLIYY
V _H H10 a-INTx	INTx	NADSYCSDSVCYENPVYDY
V _H H11 a-INTx	INTx	AAERVPSAIYTHYEFVGADEYDY
V _H H12 a-INTx	INTx	NAAGVVYDTGVYMDYTKDY
V _H H14 a-AgTx	AgTx	NSDLYTEYGQMWDL
V _H H16 a-Og XI	Og XI	AAQSPDMVRVGNFGS
V _H H18 a-KUN	KUN	EGYDGSYIDY
V _H H19 a-KUN	KUN	TADGFPYPSETMCSIPGGPDSVA
V _H H21 a-PLA ₂	PLA ₂	NLHRLTSSDDDGRT

Supplementary Table 5. Affinity measurements between V_HHs and venom fractions or toxins using BLI. x: no binding.

Toxin (sub)family	Clone	Target	K _D (M)	K _D error	K _{on} (1/Ms)	k _{on} error	K _{off} (1/s)	k _{off} error	R ²
CTx	V _H H1 a-CTx	CTx-1	x	x	x	x	x	x	x
		CTx-10	3.28·10 ⁻⁹	5.23·10 ⁻¹¹	7.42·10 ⁴	5.10·10 ²	2.44·10 ⁻⁴	3.51·10 ⁻⁶	0.99
		CTx-12	1.91·10 ⁻⁷	8.76·10 ⁻⁹	2.31·10 ⁴	1.00·10 ³	4.40·10 ⁻³	6.68·10 ⁻⁵	0.9191
		CTx-13	x	x	x	x	x	x	x
		CTx-18	8.22·10 ⁻⁹	1.03·10 ⁻¹⁰	5.64·10 ⁴	4.55·10 ²	4.64·10 ⁻⁴	4.47·10 ⁻⁶	0.9878
	V _H H2 a-CTx	CTx-10	3.81·10 ⁻⁹	4.28·10 ⁻¹¹	1.47·10 ⁵	1.25·10 ³	5.61·10 ⁻⁴	4.15·10 ⁻⁶	0.9825
		CTx-18	9.42·10 ⁻⁹	1.02·10 ⁻¹⁰	1.13·10 ⁵	1.09·10 ³	1.07·10 ⁻³	5.41·10 ⁻⁶	0.9837
	V _H H4 a-CTx	CTx-1	x	x	x	x	x	x	x
		CTx-10	x	x	x	x	x	x	x
		CTx-12	6.64·10 ⁻¹⁰	3.57·10 ⁻¹¹	1.02·10 ⁵	7.73·10 ²	6.74·10 ⁻⁵	3.58·10 ⁻⁶	0.9858
		CTx-13	3.20·10 ⁻¹⁰	5.92·10 ⁻¹²	4.18·10 ⁵	2.42·10+03	1.34·10 ⁻⁴	2.35·10 ⁻⁶	0.9854
		CTx-18	x	x	x	x	x	x	x
sNTx	V _H H5 a-sNTx	sNTx-1	1.90·10 ⁻⁹	1.41·10 ⁻¹⁰	2.49·10 ⁴	1.49·10 ²	4.72·10 ⁻⁵	3.48·10 ⁻⁶	0.9956
		sNTx-3	2.88·10 ⁻⁸	2.20·10 ⁻¹⁰	2.35·10 ⁴	1.28·10 ²	6.77·10 ⁻⁴	3.61·10 ⁻⁶	0.9965
		sNTx-5	1.69·10 ⁻⁸	1.36·10 ⁻¹⁰	3.74·10 ⁴	2.15·10 ²	6.31·10 ⁻⁴	3.58·10 ⁻⁶	0.9966
		sNTx-6	5.46·10 ⁻⁹	8.11·10 ⁻¹¹	2.98·10 ⁴	1.20·10 ²	1.63·10 ⁻⁴	2.33·10 ⁻⁶	0.9981
		sNTx-7	3.88·10 ⁻¹⁰	7.42·10 ⁻¹¹	3.71·10 ⁴	1.85·10 ²	1.44·10 ⁻⁵	2.75·10 ⁻⁶	0.9978
		sNTx-8	<1.0·10 ⁻¹²	1.16·10 ⁻¹⁰	2.24·10 ⁴	9.40·10 ¹	<1.0·10 ⁻⁷	-	0.9981
	V _H H6 a-sNTx	sNTx-9	1.70·10 ⁻⁹	7.32·10 ⁻¹¹	3.96·10 ⁴	2.11·10 ²	6.75·10 ⁻⁵	2.88·10 ⁻⁶	0.9963
		sNTx-1	9.19·10 ⁻¹⁰	8.91·10 ⁻¹¹	2.98·10 ⁴	1.38·10 ²	2.74·10 ⁻⁵	2.65·10 ⁻⁶	0.9976
		sNTx-3	6.24·10 ⁻⁸	4.27·10 ⁻¹⁰	2.56·10 ⁴	1.56·10 ²	1.60·10 ⁻³	4.97·10 ⁻⁶	0.9966
	V _H H7 a-sNTx	sNTx-8	1.36·10 ⁻⁹	1.84·10 ⁻¹⁰	2.37·10 ⁴	1.89·10 ²	3.21·10 ⁻⁵	4.34·10 ⁻⁶	0.989
		sNTx-1	4.55·10 ⁻⁹	7.39·10 ⁻¹¹	5.01·10 ⁴	3.35·10 ²	2.28·10 ⁻⁴	3.37·10 ⁻⁶	0.9967
		sNTx-3	<1.0·10 ⁻¹²	6.03·10 ⁻¹¹	8.07·10 ⁴	7.91·10 ²	<1.0·10 ⁻⁷	-	0.9825
		sNTx-8	3.37·10 ⁻⁸	3.01·10 ⁻¹⁰	5.24·10 ⁴	4.29·10 ²	1.77·10 ⁻³	6.28·10 ⁻⁶	0.9923
		sNTx-9	1.79·10 ⁻⁸	1.24·10 ⁻¹⁰	7.45·10 ⁴	4.60·10 ²	1.33·10 ⁻³	4.20·10 ⁻⁶	0.9963
INTx	V _H H9 a-INTx	INTx-3	1.18·10 ⁻⁹	1.50·10 ⁻¹¹	1.73·10 ⁵	8.88·10 ²	2.03·10 ⁻⁴	2.38·10 ⁻⁶	0.9921
		INTx-7	7.53·10 ⁻¹⁰	2.51·10 ⁻¹¹	3.60·10 ⁴	5.75·10 ¹	2.71·10 ⁻⁵	9.03·10 ⁻⁷	0.9998
	V _H H12 a-INTx	INTx-3	1.47·10 ⁻⁸	8.76·10 ⁻¹¹	8.30·10 ⁴	4.41·10 ²	1.22·10 ⁻³	3.32·10 ⁻⁶	0.9953
AgTx	V _H H13 a-AgTx	AgTx-1	7.16·10 ⁻⁹	9.88·10 ⁻¹¹	4.91·10 ⁴	3.60·10 ²	3.52·10 ⁻⁴	4.12·10 ⁻⁶	0.9946
		AgTx-2	2.16·10 ⁻⁹	6.67·10 ⁻¹¹	7.47·10 ⁴	6.68·10 ²	1.61·10 ⁻⁴	4.77·10 ⁻⁶	0.99
	V _H H14 a-AgTx	AgTx-1	7.02·10 ⁻¹⁰	5.93·10 ⁻¹¹	5.87·10 ⁴	3.79·10 ²	4.12·10 ⁻⁵	3.47·10 ⁻⁶	0.9954
		AgTx-2	7.47·10 ⁻⁹	1.84·10 ⁻¹⁰	4.27·10 ⁴	5.26·10 ²	3.19·10 ⁻⁴	6.79·10 ⁻⁶	0.9747
Og XI	V _H H15 a-Og XI	Og XI-1	2.19·10 ⁻⁹	4.10·10 ⁻¹¹	7.34·10 ⁴	3.95·10 ²	1.61·10 ⁻⁴	2.88·10 ⁻⁶	0.9963
		Og XI-2	2.16·10 ⁻⁹	5.42·10 ⁻¹¹	5.67·10 ⁴	3.08·10 ²	1.22·10 ⁻⁴	3.00·10 ⁻⁶	0.9972
	V _H H16 a-Og XI	Og XI-1	2.40·10 ⁻⁹	6.27·10 ⁻¹¹	5.51·10 ⁴	3.46·10 ²	1.32·10 ⁻⁴	3.36·10 ⁻⁶	0.9938
		Og XI-2	2.63·10 ⁻⁹	5.88·10 ⁻¹¹	6.17·10 ⁴	3.93·10 ²	1.62·10 ⁻⁴	3.48·10 ⁻⁶	0.9962
KUN	V _H H17 a-KUN	KUN-1	4.05·10 ⁻¹¹	4.13·10 ⁻¹⁰	2.08·10 ⁴	1.89·10 ²	8.43·10 ⁻⁷	8.60·10 ⁻⁶	0.9531
		KUN-2	<1.0·10 ⁻¹²	2.66·10 ⁻¹¹	8.69·10 ⁴	3.99·10 ²	<1.0·10 ⁻⁷	-	0.998
	V _H H18 a-KUN	KUN-1	2.07·10 ⁻⁹	5.39·10 ⁻¹¹	7.48·10 ⁴	5.70·10 ²	1.55·10 ⁻⁴	3.85·10 ⁻⁶	0.9875
	V _H H19 a-KUN	KUN-1	<1.0·10 ⁻¹²	3.51·10 ⁻¹¹	1.37·10 ⁵	1.53·10 ³	<1.0·10 ⁻⁷	-	0.9887
		KUN-2	1.48·10 ⁻¹⁰	1.75·10 ⁻¹¹	1.30·10 ⁵	6.44·10 ²	1.92·10 ⁻⁵	2.26·10 ⁻⁶	0.9963

Supplementary Table 6. LD₅₀s for venom fractions, toxins, and venoms using the s.c. and i.v. injection routes.

	Sample	LD ₅₀ (µg/mouse)	
		i.v.	s.c.
	INTx-7	1.3 [-]	ND
	sNTx-3	1.3 [1.3 to 1.4]	ND
<i>Dendroaspis</i>	<i>D. angusticeps</i>	38.6 [-]	41.6 [37.9 to 45.2]
	<i>D. jamesoni</i>	20.1 [-]	21.6 [20.2 to 22.9]
	<i>D. polylepis</i>	9.2 [-]	22.2 [19.1 to 25.4]
	<i>D. viridis</i>	9.8 [-]	12.9 [-]
<i>Hemachatus</i>	<i>H. haemachatus</i>	24.3 [-]	35.0 [20.4 to 24.1]
<i>Naja (Afronaja)</i>	<i>N. ashei</i>	16.7 [-]	ND
	<i>N. katiensis</i>	23.2 [-]	ND
	<i>N. mossambica</i>	22.1 [17.9 to 25.2]	ND
	<i>N. nigricincta</i>	16.5 [-]	ND
	<i>N. nigricollis</i>	17.1 [-]	79.3 [66.6 to 87.4]
	<i>N. nubiae</i>	8.7 [8.2 to 9.3]	9.6 [9.5 to 9.7]
	<i>N. pallida</i>	15.1 [-]	ND
<i>Naja (Boulengerina)</i>	<i>N. melanoleuca</i>	19.9 [-]	41.8 [-]
<i>Naja (Uraeus)</i>	<i>N. anchietae</i>	76.5 [72.3 to 80.6]	>200
	<i>N. annulifera</i>	119.2 [105.8 to 113.5]	122.6 [117.2 to 128.0]
	<i>N. haje</i>	10.1 [-]	11.9 [-]
	<i>N. nivea</i>	54.8 [54.1 to 55.5]	56.6 [53.8 to 59.2]
	<i>N. senegalensis</i>	10.1 [-]	12.2 [11.3 to 13.0]

Numbers in brackets [] represent 95% confidence intervals. [-] The confidence interval was undefined.

ND. LD₅₀ was not determined

i.v. intravenous

s.c. subcutaneous

Supplementary Table 7. In vivo experiments for the design of the recombinant antivenom

Mouse strain	Venom/toxin	V _H Hs	Molar ratio	Amount (μg)	Time of death (h)				
					M1	M2	M3	M4	M5
CD1	sNTx-3	V _H H7 a-sNTx	1:1	11.0	>24	>24	>24	-	-
CD1		V _H H5 a-sNTx	1:1	11.3	>24	>24	>24	-	-
CD1		V _H H6 a-sNTx	1:1	11.4	12	13	>24	-	-
CD1	INTx-7	V _H H9 a-INTx	1:1	9.9	8	19	>24	-	-
CD1		V _H H9 a-INTx	1:2.5	24.8	>24	>24	>24	-	-
CD1	<i>N. haje</i>	V _H H9 a-INTx	1:10	130.8	>24	>24	>24	-	-
		V _H H5 a-sNTx	1:10	134.8					
CD1	<i>N. melanoleuca</i>	V _H H9 a-INTx	1:10	286.2	>24	>24	>24	-	-
		V _H H5 a-sNTx	1:10	185.5					
CD1	<i>D. viridis</i>	V _H H9 a-INTx	1:10	293.2	0,3	>24	>24	-	-
		V _H H5 a-sNTx	1:10	224.9					
		V _H H15 a-Og XI	1:3	43.0					
CD1	<i>D. viridis</i>	V _H H9 a-INTx	1:10	293.2	>24	>24	>24	-	-
		V _H H5 a-sNTx	1:10	224.9					
		V _H H15 a-Og XI	1:3	43.0					
		V _H H13 a-AgTx	1:3	39.3					
NSA	<i>D. viridis</i>	V _H H9 a-INTx	>1:10	240.1*	>24	>24	>24	>24	>24
		V _H H5 a-sNTx	>1:10	413.1*					
		V _H H20 a-PLA ₂	>1:10	291.4*					
		V _H H1 a-CTx	>1:10	746.9*					
		V _H H4 a-CTx	>1:10	746.9*					
		V _H H17 a-KUN	>1:10	479.7*					
NSA	<i>D. jamesoni</i>	V _H H9 a-INTx	>1:10	240.1*	0,3	0,4	0,5	>24	>24
		V _H H5 a-sNTx	>1:10	413.1*					
		V _H H20 a-PLA ₂	>1:10	291.4*					
		V _H H1 a-CTx	>1:10	746.9*					
		V _H H4 a-CTx	>1:10	746.9*					
		V _H H17 a-KUN	>1:10	479.7*					
NSA	<i>D. polylepis</i>	V _H H9 a-INTx	1:10	45.4	>24	>24	>24	3 to 24	3 to 24
		V _H H5 a-sNTx	1:10	15.8					
		V _H H19 a-KUN	1:10	269.8					
		V _H H15 a-Og XI	1:10	13.8					
		V _H H13 a-AgTx	1:10	14.4					
NSA	<i>D. polylepis</i>	V _H H9 a-INTx	1:10	45.4	>24	>24	>24	>24	>24
		V _H H5 a-sNTx	1:10	15.8					
		V _H H17 a-KUN	1:10	269.8					
		V _H H15 a-Og XI	1:10	13.8					
		V _H H13 a-AgTx	1:10	14.4					
CD1	<i>N. nigricollis</i>	V _H H20 a-PLA ₂	1:10	159.9	0,3	2	>24	-	-
CD1	<i>N. nigricollis</i>	V _H H1 a-CTx	1:10	525.2	9 h	10	>24	-	-
		V _H H20 a-PLA ₂	1:10	159.9					
NSA	<i>N. nigricollis</i>	V _H H1 a-CTx	1:5	262.6	>24	>24	>24	-	-
		V _H H4 a-CTx	1:5	262.6					
		V _H H20 a-PLA ₂	1:10	159.9					

All experiments were performed by pre-incubating 3 LD₅₀ values of venom or toxin with the selected V_HHs before i.v. injection into mice.

*V_HH amount present in the recombinant antivenom.

Supplementary Table 8. Composition of the recombinant antivenom.

Toxin (sub)family	Average MW (kDa)	Venom with highest nmoles in 3 LD ₅₀ s	Abundance in venom (%)	Venom LD ₅₀ (μg/mouse)	nmoles in 3 LD ₅₀ s	Specific V _H H	nmoles of V _H H in mixture	MW of V _H H (Da)	V _H H conc. (mg/mL)	V _H H in mixture (μg)	Volume (μL/mouse)
CTx	8.0	<i>N. nigricollis</i>	77.7	17.1	4.39	V _H H1 a-CTx	43.9	17,063	29.9	746.93	25.0
CTx*					4.39	V _H H4 a-CTx	43.9	17,397	39.0	746.93	19.2
sNTx	8.0	<i>D. jamesoni</i>	31.3	20.1	2.43	V _H H5 a-sNTx	24.3	17,043	27.0	413.07	15.3
INTx	8.0	<i>N. haje</i>	40.5	10.2	1.41	V _H H9 a-INTx	14.1	17,534	29.0	240.14	8.3
AgTx	7.5	<i>D. viridis</i>	14.4	9.7	0.52	V _H H13 a-AgTx	5.2	17,336	30.0	89.18	3.0
Og XI	7.0	<i>D. jamesoni</i>	45.0	20.1	3.50	V _H H15 a-Og XI	35.0	17,069	30.0	594.6	19.8
PLA ₂	14.0	<i>N. melanoleuca</i>	27.5	19.9	1.71	V _H H20 a-PLA ₂	17.1	17,329	28.7	291.40	10.2
KUN	7.0	<i>D. polylepis</i>	74.5	9.2	2.82	V _H H17 a-KUN	28.2	17,849	28.8	479.66	16.7
Total:										3,601.9	117.3

*A second anti-CTx V_HH was added based on initial in vivo experiments with *N. nigricollis* venom.

Supplementary Table 9. Summary of in vivo experiments to assess the recombinant antivenom's efficacy to prevent venom-induced lethality.

Mouse strain	Venom sp.	Neutralizer	AV Dose (mg)	Time of death (hours)*					Notes
				M1	M2	M3	M4	M5	
Recombinant AV - Pre-incubation setup									
NSA	<i>N. haje</i>	Recombinant AV	3.6	>24	>24	>24	>24	>24	
NSA	<i>D. polytepis</i>	Recombinant AV	3.6	>24	>24	>24	>24	>24	Initial lethargy, disappeared after ~2 min for all mice
NSA	<i>D. viridis</i>	Recombinant AV	3.6	>24	>24	>24	>24	>24	
NSA	<i>N. nigricollis</i>	Recombinant AV	3.6	>24	>24	>24	>24	>24	Closed eyes, lethargy, and excessive grooming lasting over 24 hours
NSA	<i>N. nigricollis</i>	Recombinant AV	3.6	>24	>24	>24	>24	>24	Very few signs of distress or envenomation
NSA	<i>N. melanoleuca</i>	Recombinant AV	3.6	>24	>24	>24	>24	>24	Closed eyes and excessive grooming, other behavior normal
NSA	<i>D. jamesoni</i>	Recombinant AV	3.6	>24	>24	>24	>24	>24	
NSA	<i>D. angusticeps</i>	Recombinant AV	3.6	3	3	4,5	5	6,5	
NSA	<i>H. haemachatus</i>	Recombinant AV	3.6	>24	>24	>24	>24	>24	
NSA	<i>N. mossambica</i>	Recombinant AV	3.6	>24	>24	>24	>24	>24	
NSA	<i>N. pallida</i>	Recombinant AV	3.6	>24	>24	>24	>24	>24	
NSA	<i>N. ashei</i>	Recombinant AV	3.6	>24	>24	>24	>24	>24	
NSA	<i>N. katiensis</i>	Recombinant AV	3.6	>24	>24	>24	>24	>24	
NSA	<i>N. nigricincta</i>	Recombinant AV	3.6	>24	>24	>24	>24	>24	
NSA	<i>N. nubiae</i>	Recombinant AV	3.6	>24	>24	>24	>24	>24	
NSA	<i>N. anchietae</i>	Recombinant AV	3.6	>24	>24	>24	>24	>24	
NSA	<i>N. annulifera</i>	Recombinant AV	3.6	>24	>24	>24	>24	>24	
NSA	<i>N. nivea</i>	Recombinant AV	3.6	>24	>24	>24	>24	>24	
NSA	<i>N. senegalensis</i>	Recombinant AV	3.6	>24	>24	>24	>24	>24	
Commercial AV - Pre-incubation setup									
CD1	<i>D. viridis</i>	Inoserp PanAfrica	2.2	1.5	5	>24	>24	>24	
CD1	<i>N. nigricollis</i>	Inoserp PanAfrica	2.2	7	>24	>24	>24	>24	
CD1	<i>D. polytepis</i>	Inoserp PanAfrica	2.2	>24	>24	>24	>24	>24	
Recombinant AV - Rescue setup									
CD1	-	Recombinant AV		>24	>24	>24	>24	>24	
CD1	<i>N. haje</i>	Recombinant AV	4.8	>24	>24	>24	>24	>24	
CD1	<i>N. melanoleuca</i>	Recombinant AV	8.6	12	18	>24	>24	>24	Mobility impairment and haemorrhage of the eyes.
CD1	<i>D. viridis</i>	Recombinant AV	5.4	>24	>24	>24	>24	>24	
CD1	<i>D. polytepis</i>	Recombinant AV	9.9	2.5	3	2	0.75	2.2	Pain and limited mobility at 24 h. Probable haemorrhage in 2/5 mice
CD1	<i>H. haemachatus</i>	Recombinant AV	7.2	>24	>24	>24	>24	>24	
CD1	<i>D. jamesoni</i>	Recombinant AV	4.4	0.67	1.33	0.83	0.92	1.08	
CD1	<i>N. annulifera</i>	Recombinant AV	4.6	>24	>24	>24	>24	>24	
CD1	<i>N. nivea</i>	Recombinant AV	4.2	>24	>24	>24	>24	>24	
CD1	<i>N. senegalensis</i>	Recombinant AV	4.9	>24	>24	>24	>24	>24	
CD1	<i>N. nubiae</i>	Recombinant AV	4.5	>24	>24	>24	>24	>24	
CD1	<i>D. angusticeps</i>	Recombinant AV	4.4	0.83	0.30	0.40	0.25	0.33	
Commercial AV - Rescue Setup									
CD1	-	Inoserp PanAfrica	5.4	>24	>24	>24	>24	>24	
CD1	<i>N. haje</i>	Inoserp PanAfrica	2.4	>24	>24	2.2	1.7	1.7	
CD1	<i>D. polytepis</i>	Inoserp PanAfrica	5.2	1.5	>24	20	7	7	
CD1	<i>N. melanoleuca</i>	Inoserp PanAfrica	4.5	11.5	2	7.5	2.2	7.5	
CD1	<i>N. melanoleuca</i>	Inoserp PanAfrica	9.1	>24	>24	>24	>24	>24	One mouse showed severe tissue damage at the injection site
CD1	<i>H. haemachatus</i>	Inoserp PanAfrica	3.4	>24	>24	0.8	1.4	2.5	
CD1	<i>D. viridis</i>	Inoserp PanAfrica	2.8	3.8	5	12	12	24	
3LD ₅₀ Venom Control (i.v.)									
CD1	<i>D. polytepis</i>	-	-	0.5 - 0.7	0.5 - 0.7	0.5 - 0.7	-	-	
NSA	<i>D. polytepis</i>	-	-	0.18	0.22	0.28	0.33	0.42	
NSA	<i>D. viridis</i>	-	-	0.42	0.75	0.15	0.16	0.28	
NSA	<i>N. nigricollis</i>	-	-	0.15	0.15	0.14	0.16	0.29	
NSA	<i>N. melanoleuca</i>	-	-	0.05	0.05	0.2	0.22	0.25	
NSA	<i>N. haje</i>	-	-	0.08	0.14	0.17	0.15	0.19	
NSA	<i>H. haemachatus</i>	-	-	0.08	0.08	0.08	0.13	0.17	
NSA	<i>D. angusticeps</i>	-	-	0.05	0.05	0.08	-	-	
NSA	<i>D. jamesoni</i>	-	-	0.05	0.05	0.03	0.07	0.1	
NSA	<i>N. pallida</i>	-	-	<0.08	<0.08	<0.08	<0.08	<0.08	
NSA	<i>N. mossambica</i>	-	-	<0.08	<0.08	<0.08	<0.08	<0.08	
NSA	<i>N. senegalensis</i>	-	-	<0.08	<0.08	<0.08	<0.08	<0.08	
NSA	<i>M. katiensis</i>	-	-	<0.08	<0.08	<0.08	<0.08	0.13	
NSA	<i>N. ashei</i>	-	-	<0.08	<0.08	<0.08	<0.08	0.25	
NSA	<i>N. nubiae</i>	-	-	0.32	0.32	0.33	0.35	0.38	
NSA	<i>N. nigricincta</i>	-	-	0.33	0.45	0.58	0.62	2.2	
NSA	<i>N. anchietae</i>	-	-	<0.05	<0.05	<0.05	<0.05	<0.05	
NSA	<i>N. nivea</i>	-	-	<0.05	<0.05	<0.05	<0.05	<0.05	
NSA	<i>N. annulifera</i>	-	-	<0.05	<0.05	<0.05	<0.05	<0.05	
3LD ₅₀ Venom Control (s.c.)									
CD1	<i>D. polytepis</i>	-	-	0.35	0.38	0.46	0.46	0.46	
CD1	<i>N. melanoleuca</i>	-	-	0.78	0.68	1.27	1.1	0.77	
CD1	<i>N. haje</i>	-	-	0.7 - 0.8	0.7 - 0.8	0.7 - 0.8	0.7 - 0.8	0.7 - 0.8	
CD1	<i>H. haemachatus</i>	-	-	0.8 - 1.0	0.8 - 1.0	0.8 - 1.0	0.8 - 1.0	0.8 - 1.0	
CD1	<i>D. viridis</i>	-	-	0.43	0.83	0.85	0.48	0.65	
CD1	<i>D. jamesoni</i>	-	-	0.75	0.33	0.25	0.28	0.35	
CD1	<i>N. annulifera</i>	-	-	1.42	1.5	1.58	2.00	1.5	
CD1	<i>N. nivea</i>	-	-	1.5	1.5	1.5	1.5	1.5	
CD1	<i>N. senegalensis</i>	-	-	1.33	1.33	1.33	2.33	2.33	
CD1	<i>N. nubiae</i>	-	-	1.25	1.25	1.25	1.25	1.25	
CD1	<i>D. angusticeps</i>	-	-	0.58	0.25	0.22	0.23	0.33	

*M1 to M5 represent an individual mouse in each experiment

Note: The venom dose was 3 LD₅₀s

Supplementary Table 10. Data collection and refinement statistics V_HH1 a-CTx and Cardiotoxin complex. Statistics for the highest-resolution shell are shown in parentheses.

	V_HH1 a-CTx - Cardiotoxin
Wavelength (Å)	0.9762
Resolution range (Å)	51.26 - 1.6 (1.657 - 1.6)
Space group	P 2 2 21
Unit cell a, b, c (Å), α, β, γ (°)	41.94 54.90 143.01 90 90 90
Total reflections	339511 (41042)
Unique reflections	42923 (6553)
Multiplicity	7.9 (6.3)
Completeness (%)	95.87 (89.60)
Mean I/sigma(I)	9.49 (2.47)
Wilson B-factor	16.64
R-merge	0.13 (0.90)
R-meas	0.14 (0.98)
R-pim	0.05 (0.39)
CC1/2	0.996 (0.81)
Reflections used in refinement	42733 (3918)
Reflections used for R-free	2134 (195)
R-work	0.2220 (0.3172)
R-free	0.2578 (0.3480)
Number of non-hydrogen atoms	3076
Macromolecules	2782
Ligands	42
Solvent	270
Protein residues	356
R.m.s.d., bond lengths (Å)	0.008
R.m.s.d., angles (°)	1.13
Ramachandran favored (%)	97.99
Ramachandran allowed (%)	2.01
Ramachandran outliers (%)	0.00
Rotamer outliers (%)	0.66
Clashscore	8.50
Average B-factor (Å²)	20.98
Macromolecules	20.25
Solvent	32.80

Supplementary Table 11. Data collection and refinement statistics V_HH5 a-sNTx and sNTx1 complex. Statistics for the highest-resolution shell are shown in parentheses.

	V_HH5 a-sNTx – sNTx1
Wavelength (Å)	0.72931
Resolution range (Å)	23.29 - 2.5 (2.589 - 2.5)
Space group	C 2 2 21
Unit cell a, b, c (Å), α, β, γ (°)	104.15 104.09 79.34 90 90 90
Total reflections	206365 (33854)
Unique reflections	28844 (4687)
Multiplicity	7.15 (7.22)
Completeness (%)	99.9 (100.00)
Mean I/sigma(I)	19.86 (3.67)
Wilson B-factor	62.89
R-merge	0.056 (0.498)
R-meas	0.06 (0.537)
R-pim	0.02 (0.20)
CC1/2	0.999 (0.929)
Reflections used in refinement	15225 (1502)
Reflections used for R-free	760 (75)
R-work	0.3238 (0.4378)
R-free	0.3615 (0.5450)
Number of non-hydrogen atoms	2903
Macromolecules	2852
Ligands	16
Solvent	35
Protein residues	376
R.m.s.d., bond lengths (Å)	0.005
R.m.s.d., angles (°)	0.90
Ramachandran favored (%)	86.68
Ramachandran allowed (%)	13.32
Ramachandran outliers (%)	0.00
Rotamer outliers (%)	0.62
Clashscore	12.87
Average B-factor (Å²)	71.01
Macromolecules	70.94
Solvent	81.85