The Homologous Relationship of Ophidian Amino Acids, as it Would Apply to a DNA-Based Vaccine in Humans

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Part of my responsibility with this vaccine was to track down the complete DNA sequences of snake venom fractions that I was to use on myself, or humans in the field. This was a large task due to the plethora of deleterious fractions that venomous snake have. There are about 500 species of venomous snakes, and the biggest killers are Elapidae/Viperidae(3).Of those, only a handful are dangerous to humans due to human populations that encounter these snakes, venom toxicity, aggression, etc. Certain venomous snakes can have over 40-50 deleterious fractions that can be harmful to humans. How do you pick from this selection to chose the ones that we will use for a DNA-based vaccine protocol? Two more very important goals I aimed at achieving where finding what other snake venom fractions had similar amino acid sequences, and what amino acids were involved in the epitope for IgG binding and possible cross-reactivity, that might be important with developing the DNA-based vaccine for humans. I felt a thorough epitope mapping mission was in order to reveal these critical IgG binding sites to maybe make a better vaccine.

This is where I turned to a super article by J.P. Chippaux on The Global Evaluation of Snakebites(1). His in depth article helped me focus in on the major culprits of snakebite death throughout the world. This gave me a great picture for the overall crisis of snakebite death. I couldn't start to build my theoretical case for a DNA-based vaccine without the appropriate fractions that would save the most amount of lives, fingers, hands, legs, etc. His study showed these results on snakebite death throughout the world. Europe(30 deaths), Middle East(100 deaths), USA/Canada(15 deaths), Central/South America(5,000 deaths), Africa(20,000 deaths), Asia(100,000 deaths), and Oceania(200)(1).

At this point I studied the major killers in these parts of the world and made a detailed list of them to further break down the schematic of death. South American deaths a year are due to Bothrops mainly(atrox, asper, jararaca,etc.). Crotalus durissus also comes into play, but Bothrops is the major killer.

Africa is next with many genera that can kill. Echis has 9 spp. that cause human deaths(coloratus, hughesi, jogeri, leucogaster, megalocephalus, ocellatus, omanensis, and pyramidum[3]). Bitis has 16 species that cause human deaths(albanica, arietans, armata, atropos, caudalis, cornuta, gabonica, heraldica, inornata,nasicornis, parviocula, peringueyi, rubida, schneideri, worthington, xeropaga[3]). Naja has 10 spp that cause human deaths(haje, melanoleuca, mossambica, katiensis, pallida, nigricollis, nivea, anchietae, annulifera, and nubiae[3]). The Dendroaspis genus has four species that cause human deaths (angusticeps, jamesoni, polylepis, and viridis[3]).

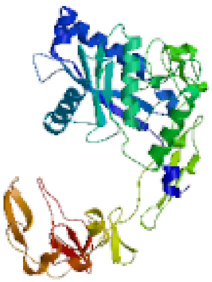
Asia has the biggest ratio of snakebite death due to the big four(plus others). Indian cobra, common krait, russell's viper, and the saw-scaled viper. The cobras consist of Naja(atra, kaouthia, mandalayensis, naja, oxiana, philippinensis, sagittifera, samaras, siamensis, sputatrix, and sumatrana[3]). Bungarus has (andamanensis, bungaroides, caeruleus, candidus, ceylonicus, fasciatus, flaviceps, lividus, magnimaculatus, niger, sindanus, Lewinski[3]).
Daboia russelii, and Echis carinatus also have very large roles in snakebite death in Asia.

At this time I focused on the major killers, with the major fractions that cause death or disability. I've chosen these genera, with these certain fractions that cause the most harm.

I broke it down into these major families that create the most trouble: SVMP(causes haemorrhage), PLA2(pro/anti coagulant), CTL(platelet/coagulation problems) alpha three-finger postsynaptic neurotoxins(inhibits nAChR and produces peripheral paralysis at the NMJ),PLA2 presynaptic beta neurotoxins(blocks acetylcholine release at the nerve terminal[2,4]). There are many more, but this is a strong representation of deleterious fractions from multiple genera as a comparison. The Harrisson paper also uses SVMP, PLA2 and CTL as a amino acid comparison(2).

SVMP Amino Acid Comparisons

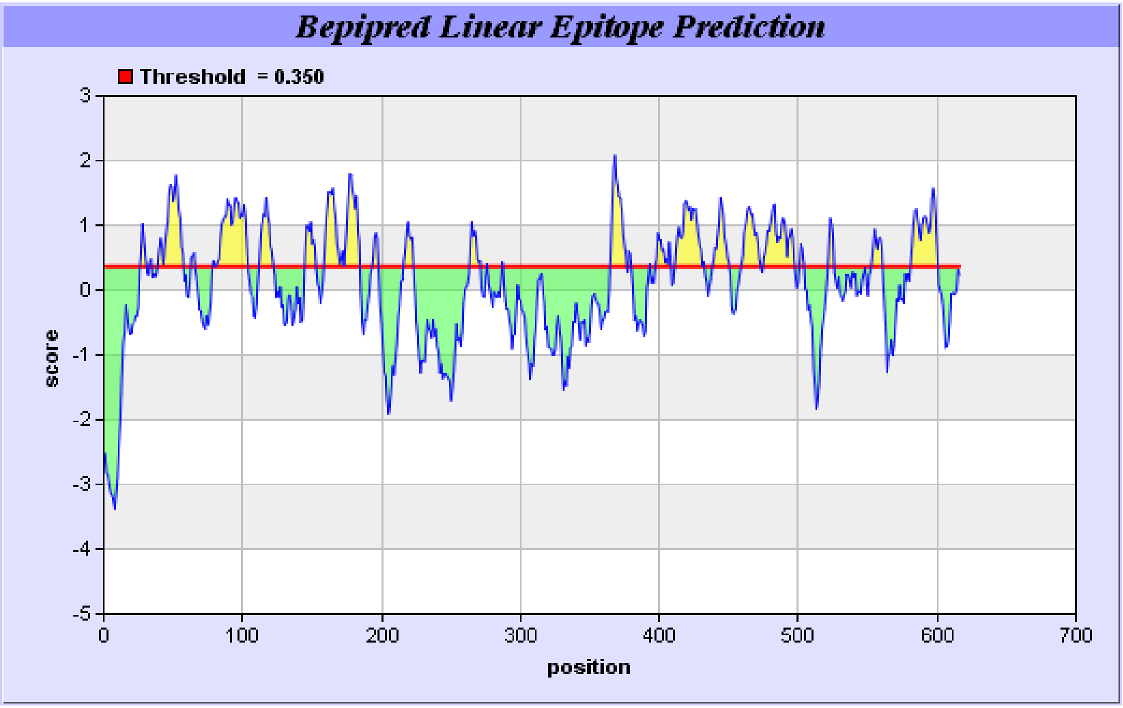
Kenyan Echis carinatus(ecarin, Q90495)616aa, mature protein 426aa(90% identity with Echis ocellatus fraction EoMP06[4]).



(ref. 5, 6)

No.	Start Position	End Position	Peptide	Peptide Length
1	26	30	GNVND	5
2	33	34	VV	2
3	39	57	VTALPKGAVQQPEQKYEDA	19
4	63	65	EVK	3
5	79	103	LFSEDYSETHYSSDDREITTNPSVE	25
6	112	122	IQNDAESTASI	11
7	145	152	KIPDSEAH	8
8	159	184	NIENEDEAPKMCGVTQDNWESDEPIK	26
9	194	197	HERK	4
10	216	223	KYNNDSTA	8
11	264	271	STADDTLH	8
12	276	276	W	1
13	287	287	H	1
14	365	376	ESIPPPKEFSSC	12
15	378	379	YD	2
16	393	393	I	1
17	397	408	PLRKDIASPAVC	12
18	412	432	IWEEGEECDCGSPADCRNPCC	21
19	438	450	KLKPGAECGNGEC	13
20	459	473	AGTECRPARDDCDVA	15
21	475	497	HCTGQSAECPRNEFQRNGQPCLN	23

22	501	504	YCYN	4
23	522	525	ATVA	4
24	548	548	Y	1
25	553	560	FPCAPQDV	8
26	581	600	DYSYADENKGIVEPGTKCED	20



Reference: Jens Erik Pontoppidan Larsen, Ole Lund and Morten Nielsen. Improved method for predicting linear B-cell epitopes. Immunome Res. 2006; 2: 2.

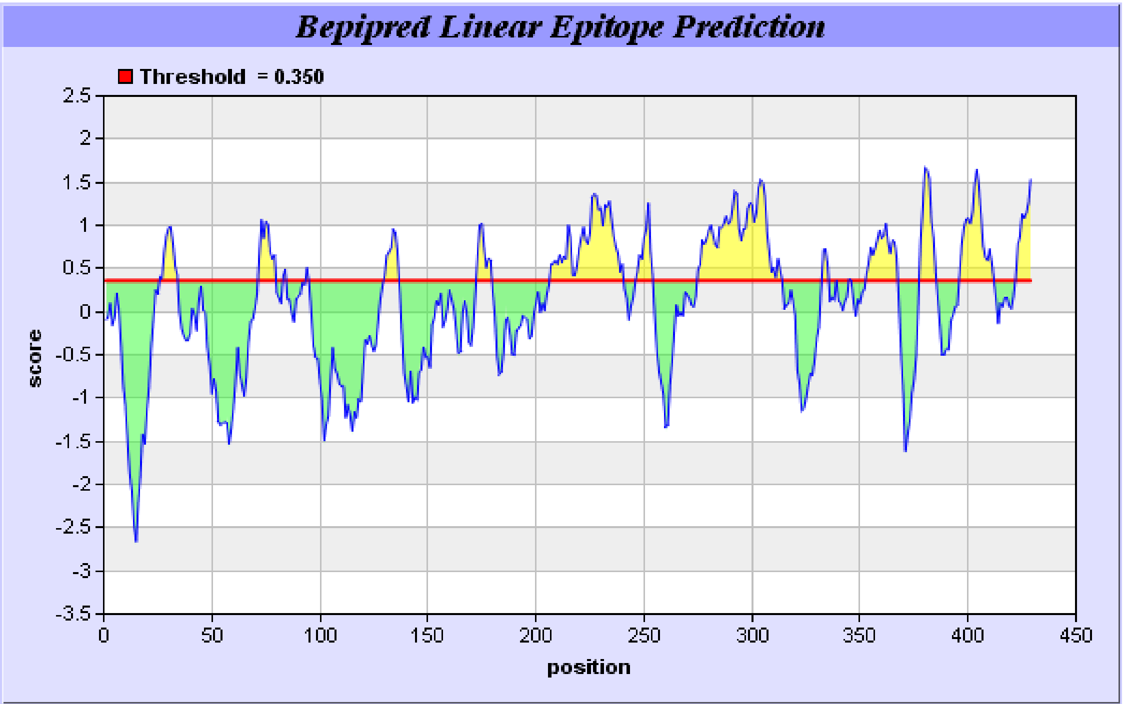
MIQILLVIICLAVFPYQGCSIILGSGNVNDYEVVYPQKVTALPK
GAVQQPEQKYEDAMQYEFEVKGEPVVLHLEKNKELFSEDYSETHYSSDDREITT
NPSV
EDHCYYHGRIQNDAESTASISACNGLKGHFKLRGETYFIEPLKIPDSEAHAVYKY
ENI
ENEDEAPKMCGVTQDNWESDEPIKKTLGLIVPPHERKFEKKFIELVVVVDHSMV
TKYN
NDSTAIRTWIYEMLNTVNEIYLPFNIRVALVGLEFWCNGDLINVTSTADDTLHSF
GEW
RASDLLNRKRHDHAQLLTNVTLDHSTLGITFVYGMCKSDRSVELILDYSNITFNM
AYI
IAHEMGHSLGMLHDTKFCTCGAKPCIMFGKESIPPPKEFSSCSYDQYNKYLLKYN
PKC
ILDPPLRKDIASPAVCGNEIWEEGEECDCGSPADCRNPCCDAATCKLKPGAECGN
GEC
CDKCKIRKAGTECRPARDDCDVAEHCTGQSAECPRNEFQRNGQPCLNNSGYCY
NGDCP
IMLNQCIALFSPSATVAQDSCFQRNLQGSYYGYCTKEIGYYGKRFPCAPQDVKC
GRLY
CLDNSFKKNMRCKNDYSYADENKGIVEPGTKCEDGKVCINRKCVDVNTAY

Daboia russellii(RVV-X, Q7LZ61),429aa(4)

(ref. 5, 6)

No.	Start Position	End Position	Peptide	Peptide Length
1	26	34	KCNSTATNT	9
2	72	79	SSADETLN	8
3	83	84	EW	2
4	94	94	S	1
5	130	136	QEQGNRN	7
6	173	179	LSDQPSK	7
7	207	240	RKDIVSPPVCGNEIWEEGEECDCGSP ANCQNPCC	34

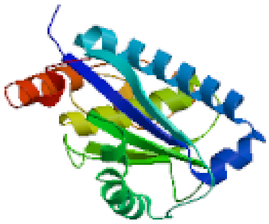
8	246	254	KLKPGAECG	9
9	275	314	RARDECDVPEHCTGQSAECPRDQLQ QNGKPCQNNRGYCYN	40
10	333	335	NVA	3
11	339	339	C	1
12	345	346	KG	2
13	353	367	RKENGRKIPCAPQDV	15
14	378	385	SPRNKNPC	8
15	396	411	KGMVDPGTKCEDGKVC	16



L V S T S A Q F N K I F I E L V I I V D H S M A K K C N S T A T N T K I Y E I V N S A N E I F N P L N I H V T L I G V E F W C D R D L I N V T S S A D E T L N S F G

E W R A S D L M T R K S H D N A L L F T D M R F D L N T L G I T F L A G M C Q
A Y R S V E I V Q E Q G N R N F K T A V I M A H E L S H N L G M Y H D G K N
C I C N D S S C V M S P V L S D Q P S K L F S N C S I H D Y Q R Y L T R Y K P K
C I F N P P L R K D I V S P P V C G N E I W E E G E E C D C G S P A N C Q N P C
C D A A T C K L K P G A E C G N G L C C Y Q C K I K T A G T V C R T R R A R D
E C D V P E H C T G Q S A E C P R D Q L Q Q N G K P C Q N N R G Y C Y N G D
C P I M R N Q C I S L F G S R A N V A K D S C F Q E N L K G S Y Y G Y C R K E
N G R K I P C A P Q D V K C G R L F C L N N S P R N K N P C N M H Y S C M D
Q H K G M V D P G T K C E D G K V C N N K R Q C V D V N T A Y Q S T T G

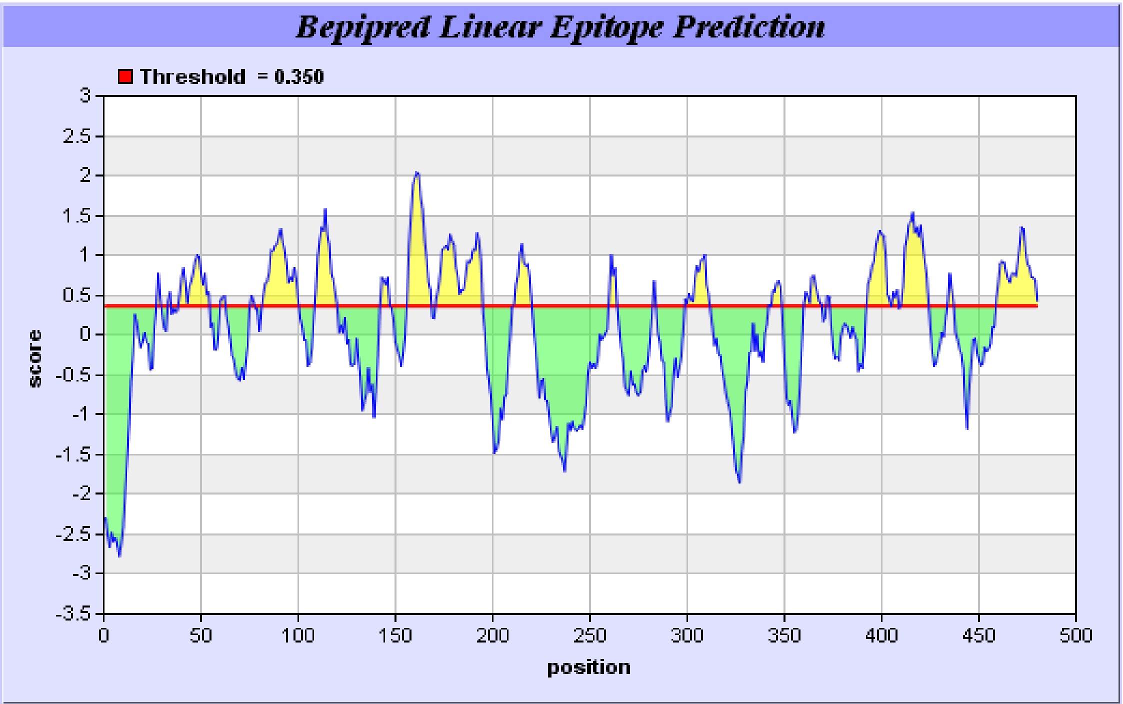
Trimeresurus gramineus(trigram in, P15503),480aa(4)



(ref. 5, 6)

No.	Start Position	End Position	Peptide	Peptide Length
1	28	29	LN	2
2	33	34	VV	2
3	39	54	VTALPKGAVQQKYEDA	16
4	60	62	KVN	3
5	76	77	LF	2
6	82	100	SEIHYSPDGREITAYPSVE	19
7	109	120	IENDADSTASIS	12
8	143	147	LSDSE	5
9	156	168	NVEKEDEPPKMCG	13
10	171	194	QNWESYESTKKASQLNVTPEQQRF	24
11	210	220	YTKYSGNSERI	11
12	260	264	ASAPT	5
13	283	284	HD	2

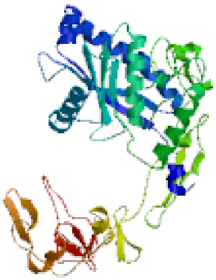
14	299	311	IGRAPVGGMCDPK	13
15	342	342	H	1
16	344	348	DEDKC	5
17	360	369	SRQPSKYFSE	10
18	372	373	KD	2
19	393	408	RTDTVSTPVSGNELLE	16
20	410	424	GEDCDCGSPANPCCD	15
21	434	437	AQCG	4



MIQVLLITICLAVFPYQGSSIILESGNLNDYEVVYPEKVTALPK
GAVQQKYEDAMQYEFKVNGEPVVLHLEKNKGLFSEDYSEIHYSPDGREITAYPS

VEDH
CYYHGRIENDADSTASISACDGLKGHFKLQGEMYLIEPLELSDSEAHAVFKYENV
EKE
DEPPKMCGVTQNWESYESTKKASQLNVTPEQQRFPQRYIKLGIFVDHGMYTKYS
GNSE
RITKRVHQMINNINMMCRALNIVTTLSVLEIWSEKDLITVQASAPTTLTLFGAWR
ETV
LLNRTSHDHAQLLTATIFNGNVIGRAPVGGMCDPKRSVAIVRDHNAIVFVVAVT
MTHE
MGHNLGMHHDEDKCNCNTCIMSKVLSRQPSKYFSECSKDYYQTFLTNHNPQCIL
NAPL
RTDTVSTPVSGNELLEAGEDCDCGSPANPCCDAATCKLIPGAQCGEGLCCDQCSF
IEE GTVCRIARGDDLDDYCNGRSAGCPRNPFHA

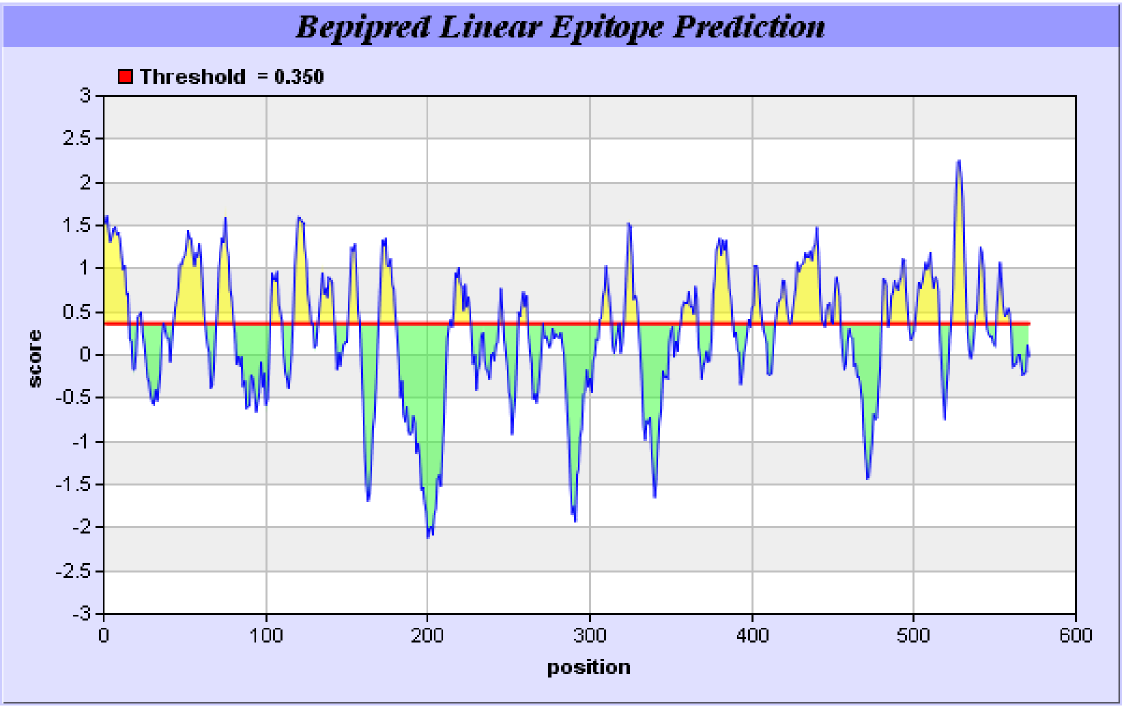
Bothrops jararaca(jararhagin, P30431), 571aa(90% identity with Bothrops jararaca Bothropasin, Bothrops insularis, and Crotalus d.d. SVMP P-III[4]).



(ref. 5, 6)

No.	Start Position	End Position	Peptide	Peptide Length
1	1	15	ATRPKGAVQPKYEDA	15
2	21	23	KVN	3
3	37	37	L	1
4	43	62	SEIHYSPDGREITTYPPVED	20
5	70	80	IENDADSTASI	11
6	103	110	KLPDSEAH	8
7	117	129	NVEKEDEAPKMCG	13
8	133	142	NWKSYEPIKK	10
9	151	158	EQQRYDPY	8
10	170	180	GTVTKNNGDLD	11

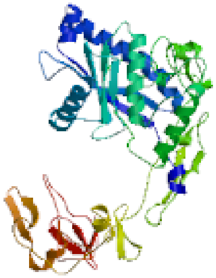
11	214	214	N	1
12	216	226	DKITVKPDVDY	11
13	244	246	KHD	3
14	256	261	FNGPTI	6
15	271	271	H	1
16	306	313	DTGSCSCG	8
17	318	318	I	1
18	321	329	PTISNEPSK	9
19	356	366	LGTDIISPPVC	11
20	376	388	ECDCGTPENCQNE	13
21	398	398	K	1
22	400	406	GSQCGHG	7
23	415	444	SKSGTECRASMSECDPAEHCT GQSSECPAD	30
24	446	454	FHKNGQPCL	9
25	480	483	VYEA	4
26	485	496	DSCFKDNQKGNY	12
27	501	515	RKENGKKIPCAPEDV	15
28	523	532	KDNSPGQNNP	10
29	538	544	SNDDEHK	7
30	551	559	TKCADGKVC	9



Reference: Jens Erik Pontoppidan Larsen, Ole Lund and Morten Nielsen. Improved method for predicting linear B-cell epitopes. Immunome Res. 2006; 2: 2.

ATRPKGAVQPKYEDAMQYEFKVNGEPVVLHLEKNKGLFSKDYSE
IHYSPDGREITTYPPVEDHCYYHGRIENDADSTASISACNGLKGYFKLQRETYFIE
PL
KLPDSEAHAVFKYENVEKEDEAPKMCGVTQNWKSYEPIKKASQLAFTAEQQRY
DPYKY
IEFFVVVDQGTVTKNNGDLDKIKARMYELANIVNEIFRYLYMHVALVGLEIWSN
GDKI
TVKPDVDYTLNSFAEWRKTDLLTRKKHDNAQLLTAIDFNGPTIGYAYIGSMCHP
KRSV
GIVQDYSPINLVVAVIMAHEMGHNLGIHHDTGSCSCGDYPCIMGPTISNEPSKFFS
NC
SYIQCWDFIMNHNPECIINEPLGTDIISPPVCGNELLEVGEECDCGTPENCQNECC
DA
ATCKLKSGSQCGHGDCCEQCKFSKSGTECRASMSECDPAEHCTGQSSECPADVF
HKNG
QPCLDNYGYCYNGNCPIMYHQCYALFGADVYEAEDSCFKDNQKGNYYGYCRK
ENGKKI
PCAPEDVKCGRLYCKDNSPGQNNPCKMFYSNDDEHKGMVLPGTKCADGKVCS
NGHCVD VATAY

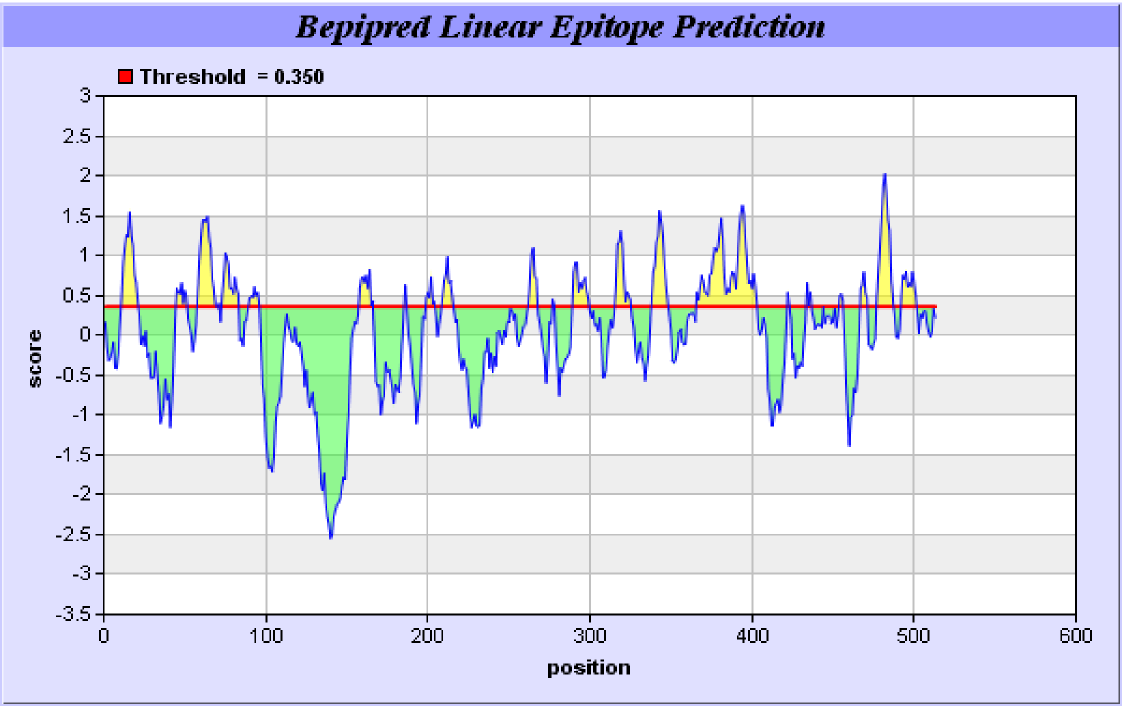
Bitis arietans(BA-MP-2, Q4QT06), 513aa(4)



(ref. 5, 6)

No.	Start Position	End Position	Peptide	Peptide Length
1	11	21	IQNDADSTASI	11
2	45	51	LSDSEAH	7
3	58	71	NIEKEDEAPKMCGV	14
4	73	83	QTTWESDLPIK	11
5	90	96	ATSEQQK	7
6	158	166	QINVQSAPS	9
7	186	187	HD	2
8	199	204	GSTVGI	6
9	209	215	GMCDPKR	7
10	262	267	ANAGPQ	6
11	277	278	WN	2
12	290	299	KCIDNEPLRT	10
13	316	325	EECDCGPSKI	10
14	338	348	LTPGSQRSHGE	11
15	366	403	RSECDLPEHCTGQSAECPSDVFKRN GQPCQNNNGYCYN	38
16	422	423	TV	2
17	434	436	KGT	3

18	454	456	QDM	3
19	467	471	SIGNT	5
20	477	487	YSSQDDPDYGM	11
21	492	501	TKCEDGKVCN	10



Reference: Jens Erik Pontoppidan Larsen, Ole Lund and Morten Nielsen. Improved method for predicting linear B-cell epitopes. Immunome Res. 2006; 2: 2.

VEDHCYYHGRIQNDADSTASISACNGLKGHFKLRGEMYLIEPLK
LSDSEAHAVYKYENIEKEDEAPKMCGVTQTTWESDLPIKKASQLVATSEQQKVF
NKFI
EIYMIADNVTFNKYSRSLTAVRTRMYEILNDLNLIFIQFRIYIALIGLEIWSTNNQIN
VQSAPSVTLRLFEDWRQNVLLKRKRHDNAQLLTVVDLDGSTVGIAPLGGMCDP
KRSVG
LVQDYCKSHILVAATMAHELGHSLGIKHDNVNCTCNGRPCIMSANAGPQRVFSF
SDCS
WNQYQKFLVDHKPKCIDNEPLRTDIVSPAVCGNHFLEKEEECDCGPSKICRNPCC
NAV

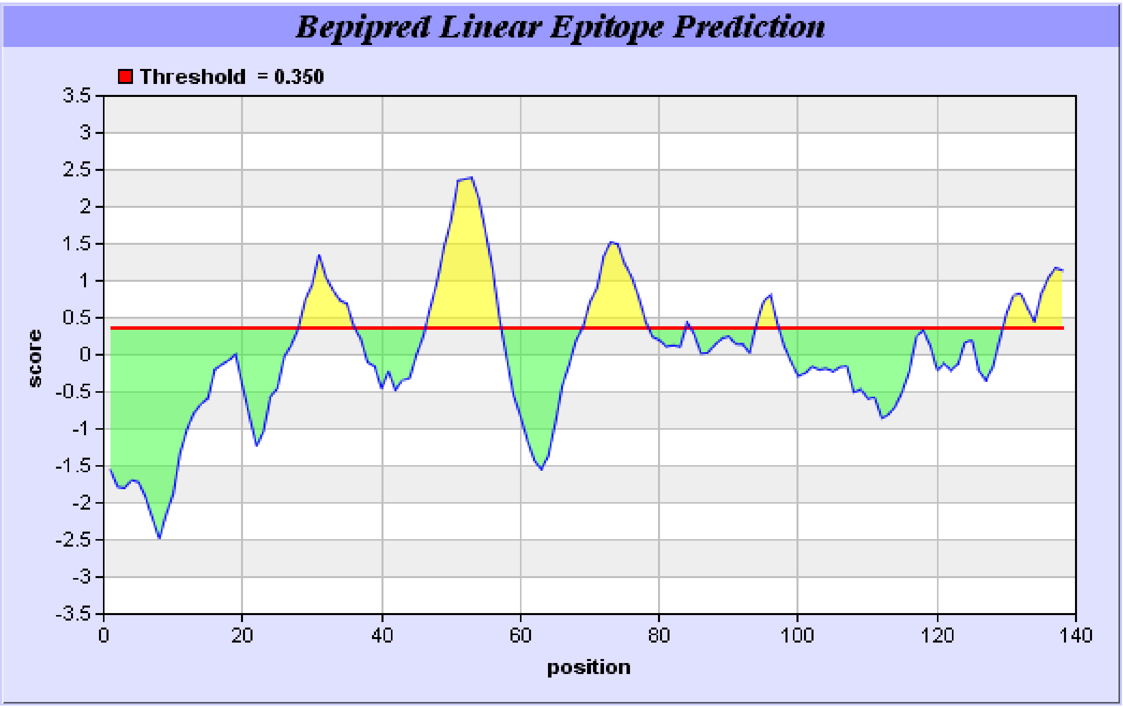
TCKLTPGSQRSHGECCDQCRIKTAGTVCRRERSECDLPEHCTGQSAECPSDVFKR NGQ
PCQNNNGYCYNGDCPILTNQCISLFGSRVTVAPDVCFTLNKKGTNKFHCRKENG RYIK
CAPQDMKCGRLFCVEPSIGNTVGCKFYSSQDDPDYGMVDLGTKCEDGKVCNSN RECVD VNTAY

PLA2(Pro/Anticoagulant)Amino Acid Comparisons

Bitis arietans(BA-PLA2-32, Q4QT03), 138aa(100% identity with Echis ocellatus PLA2-5, and 90% identity with Bitis arietans PLA2-19[4]).

(ref. 5, 6)

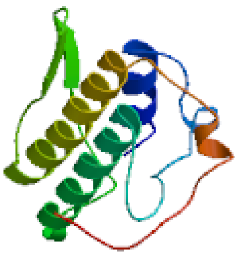
No.	Start Position	End Position	Peptide	Peptide Length
1	28	36	ETGRSPFPF	9
2	47	57	GGKGKPKDDTD	11
3	69	78	SMPDCSPKTD	10
4	84	84	R	1
5	94	97	GTSC	4



Reference: Jens Erik Pontoppidan Larsen, Ole Lund and Morten Nielsen. Improved method for predicting linear B-cell epitopes. Immunome Res. 2006; 2: 2.

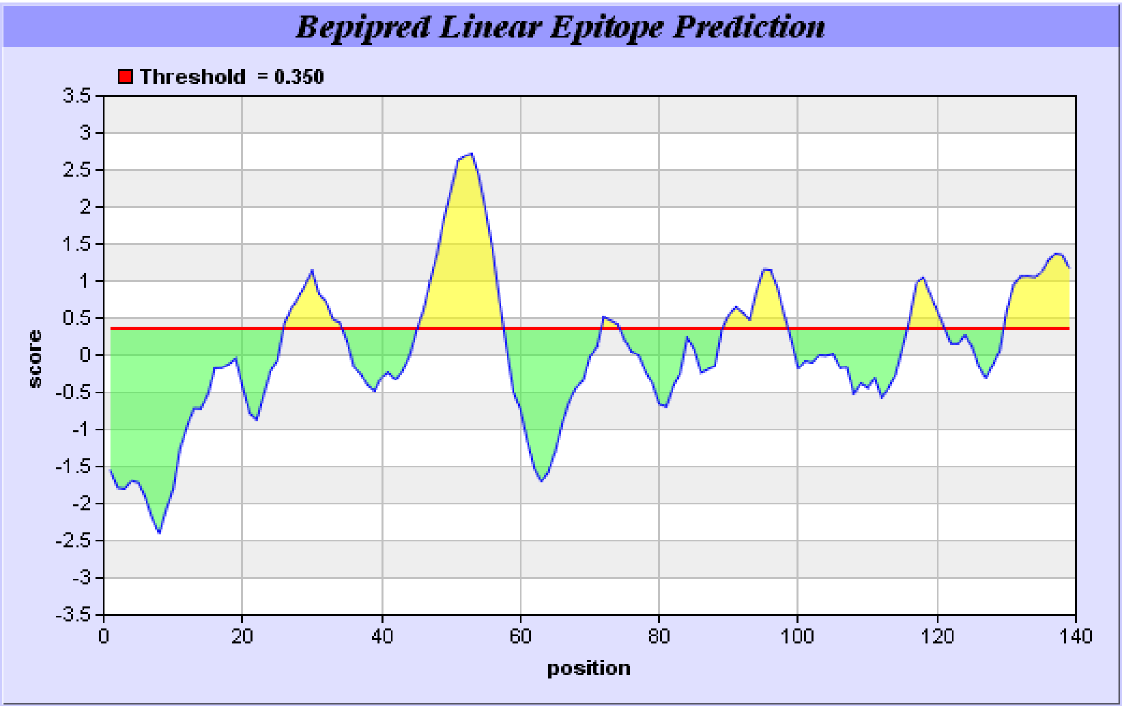
MRTLWIVAVWLIGVEGSVIEFGTMIIEETGRSPFPFYTSYGCYC
GLGGKGKPKDDTDRCCFVHDCCYGSMPDCSPKTDIYRYHRENGEIICESGTSCEK
RIC ECDKAAAVCFRENLKTYKNKYMVYPDSLCKEESEKC

Echis pyramidum leakyi(EplPLA2-5, P59172), 139aa(100% identity with Bitis arietans PLA2-20, 90% identity with Echis coloratus sochureki PLA2-4, and Bitis arietans PLA2-18[4]).



(ref. 5, 6)

No.	Start Position	End Position	Peptide	Peptide Length
1	26	34	KNKTGKPAM	9
2	46	57	WGGQGKPQD PSD	12
3	72	74	NCS	3
4	89	98	IICGDNDPCR	10
5	116	121	VNTYDE	6

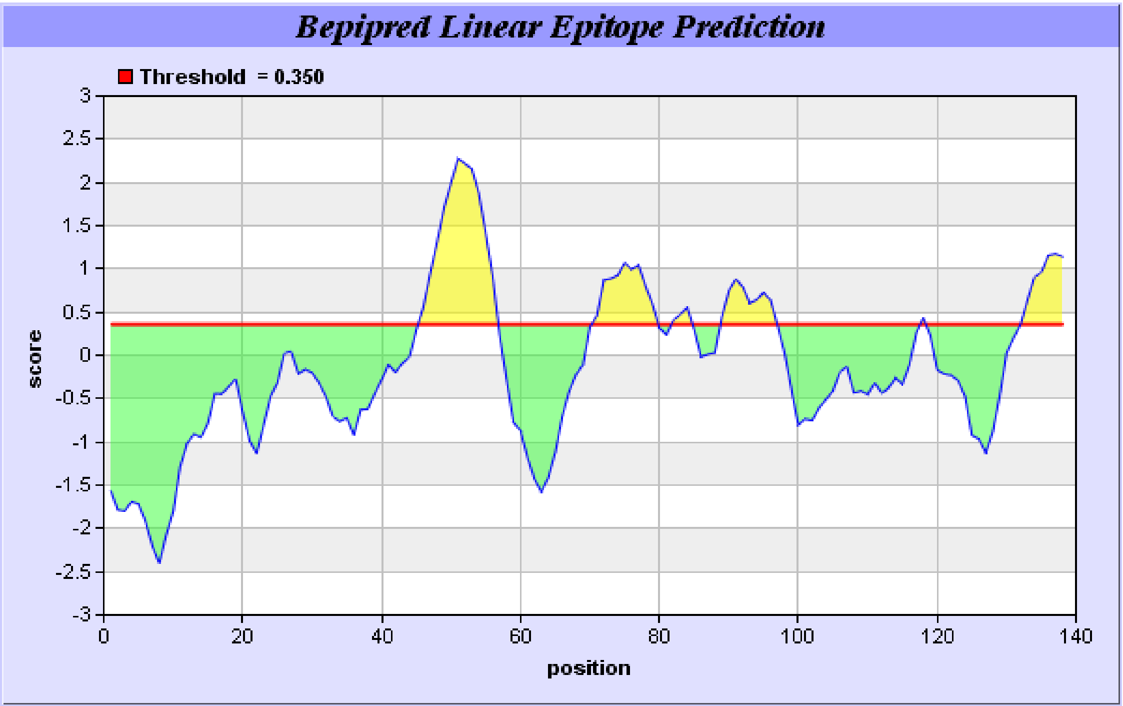


Reference: Jens Erik Pontoppidan Larsen, Ole Lund and Morten Nielsen. Improved method for predicting linear B-cell epitopes. Immunome Res. 2006; 2: 2.

MRTLWIVAVWLMGVEGNLYQFGKMIKNKTGKPAMFSYSAYGCYC
GWGGQGKPQDPSDRCCFMHDCCYTRVNNCSPKMTLYSYRFENGDIICGDNDPC
RKAVC ECDREAAICLGENVNTYDEKYRFYSSSYCTEEESEKC

Echis coloratus(EC, Q910A0), 138aa(50% identity with over 50 different fractions[4])

No.	Start Position	End Position	Peptide	Peptide Length
1	46	56	WGGKGKPQ DAT	11
2	71	79	NGCDPKMGT	9
3	82	84	YSF	3
4	89	97	IVCGGDDPC	9
5	118	118	T	1

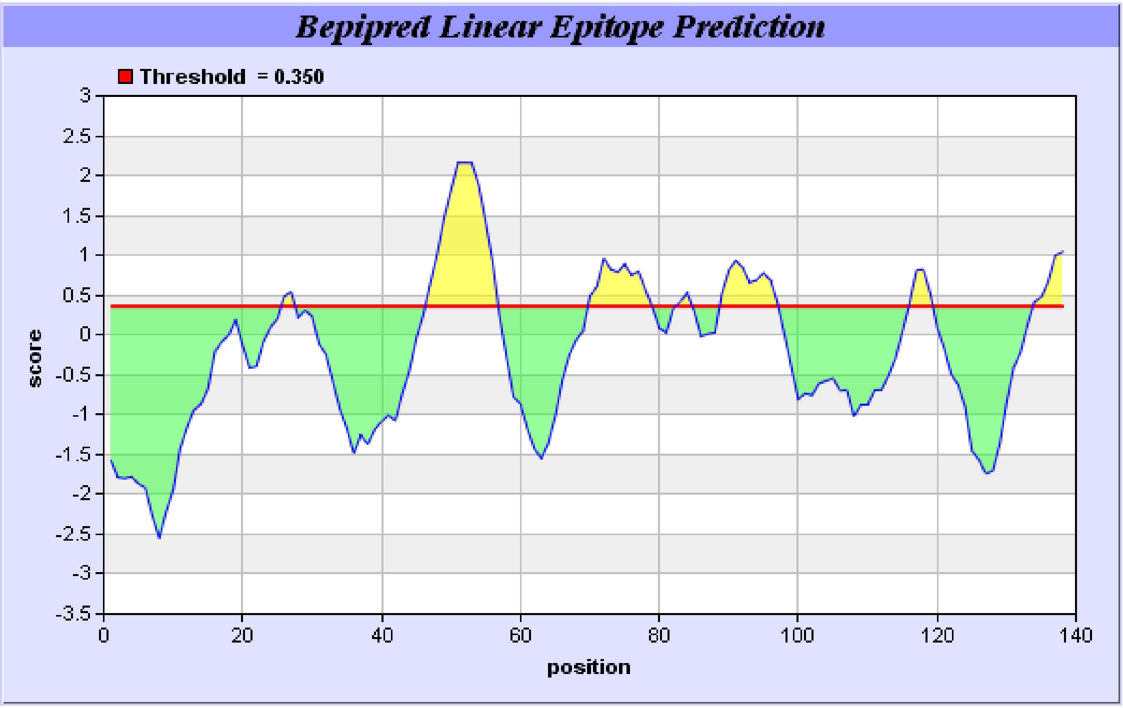


Reference: Jens Erik Pontoppidan Larsen, Ole Lund and Morten Nielsen. Improved method for predicting linear B-cell epitopes. Immunome Res. 2006; 2: 2.

MRTLWIVAVWLMGVEGHLYQFENMIYQKTGKFAIIAYSNYGCYC
GWGGKGKPQDATDRCCFVHDCCYGRVNGCDPKMGTYSYSFQNGDIVCGGDDP
CLRAVC ECDRVAANCFAENLKTYNKKYWLSSIIDCKEESEKC

Vipera palaestinae(VP, Q98996), 138aa(synergistically lethal with VP 7&8[4]).

No.	Start Position	End Position	Peptide	Peptide Length
1	26	27	NK	2
2	47	56	GGKGKPQDAT	10
3	70	78	VNGCDPKLS	9
4	83	84	SF	2
5	89	97	IVCGDDDPC	9
6	116	119	MNTY	4

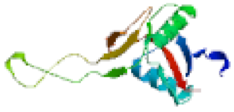


Reference: Jens Erik Pontoppidan Larsen, Ole Lund and Morten Nielsen. Improved method for predicting linear B-cell epitopes. Immunome Res. 2006; 2: 2.

MRTLWIVAVCLMGVEGHLTQFGDMINKKTGTFGLLSYVYYGCYC
GLGGKGKPQDATDRCCFVHDCCYGTVNGCDPKLSTYSYSFQNGDIVCGDDDPC
LRAVC ECDRVAAICFGENMNTYDTKYMLHSLFDCMEESEKC

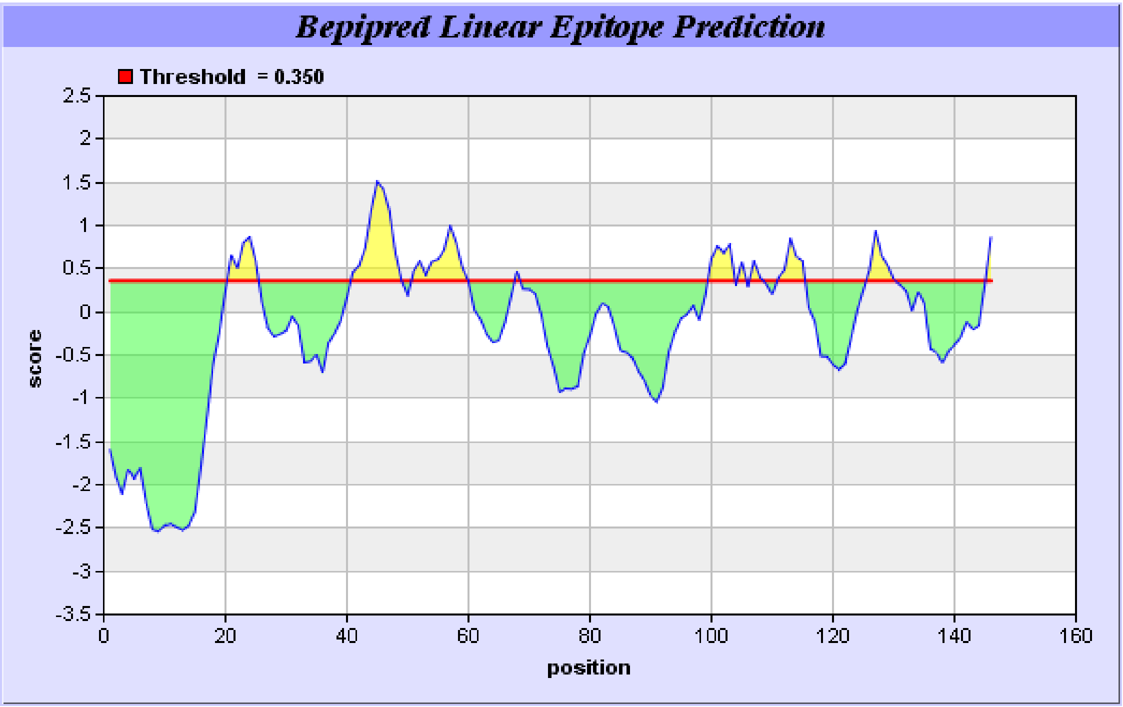
CTL Amino Acid Comparisons

Bitis arietans(CTL-1, Q6X5T5), 146aa(90% identity with Echis carinatus[Carinactivase-1], Echis carinatus sochureki[CTL-9], Echis pyramidum leaky[CTL-5][4]).



(ref. 5, 6)

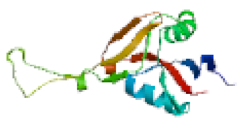
No.	Start Position	End Position	Peptide	Peptide Length
1	21	25	TEADC	5
2	41	49	DEPKTWADA	9
3	51	60	KFCSEQANGG	10
4	68	68	R	1
5	100	103	WGWT	4
6	105	105	G	1
7	107	108	KL	2
8	111	115	EARAE	5
9	126	130	KKEWK	5



Reference: Jens Erik Pontoppidan Larsen, Ole Lund and

MGRFIFISFGLLVVFLSLSGTEADCLPDWFHYEGHCYRVFDEPK
TWADAEKFCSEQANGGHLVSVHSRKEAGLVGVLAYQTLESPIVWMGLSKVWN
QCDWGW TNGAKLKYEARAEESYCIHITSKKKEWKSLPCRNYGHFVCKSPA

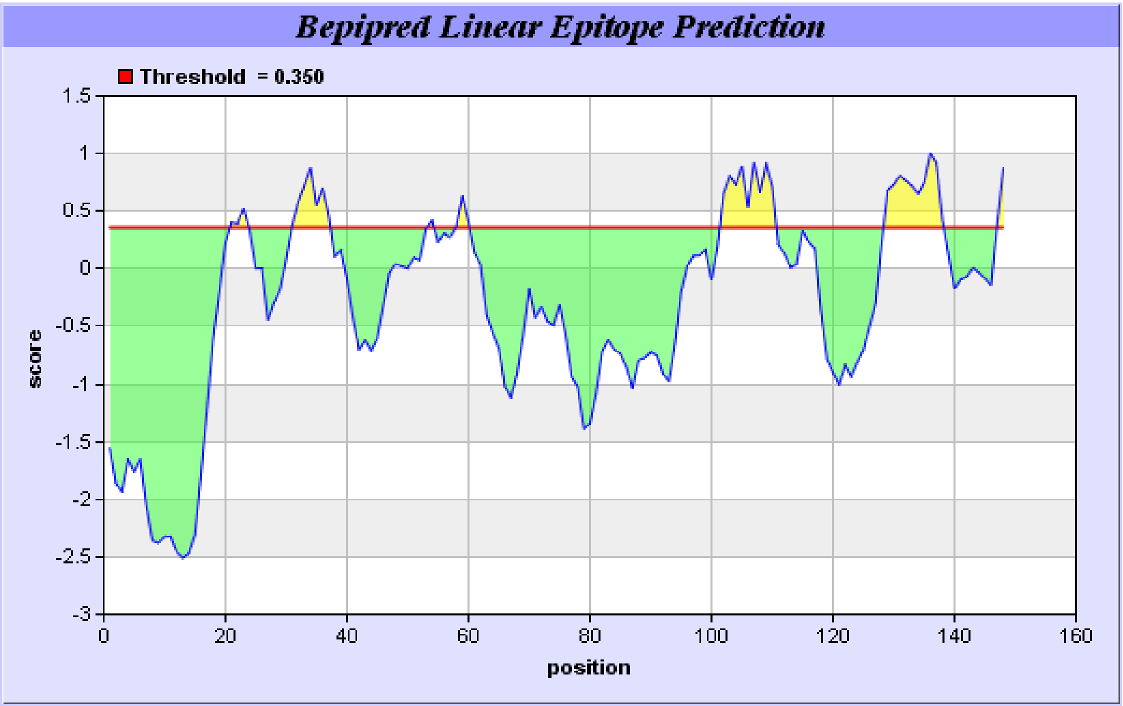
Echis pyramidum leakeyi(CTL-1, Q6X5S3), 148aa(100% identity with Echis carinatus sochureki[CTL-1], Bitis arietans[CTL-8], Echis ocellatus[CTL-2], and 90% identity with Echis c.s.[CTL-1], Bitis arietans[CTL-6&8], and Echis ocellatus[CTL-1&2][4]).



(ref. 5, 6)

No.	Start Position	End Position	Peptide	Peptide Length
1	21	23	TEA	3

2	31	37	WSGYDQN	7
3	54	54	F	1
4	58	60	QHK	3
5	102	110	WGWTDGA QL	9
6	129	138	NHWSRTDC SG	10



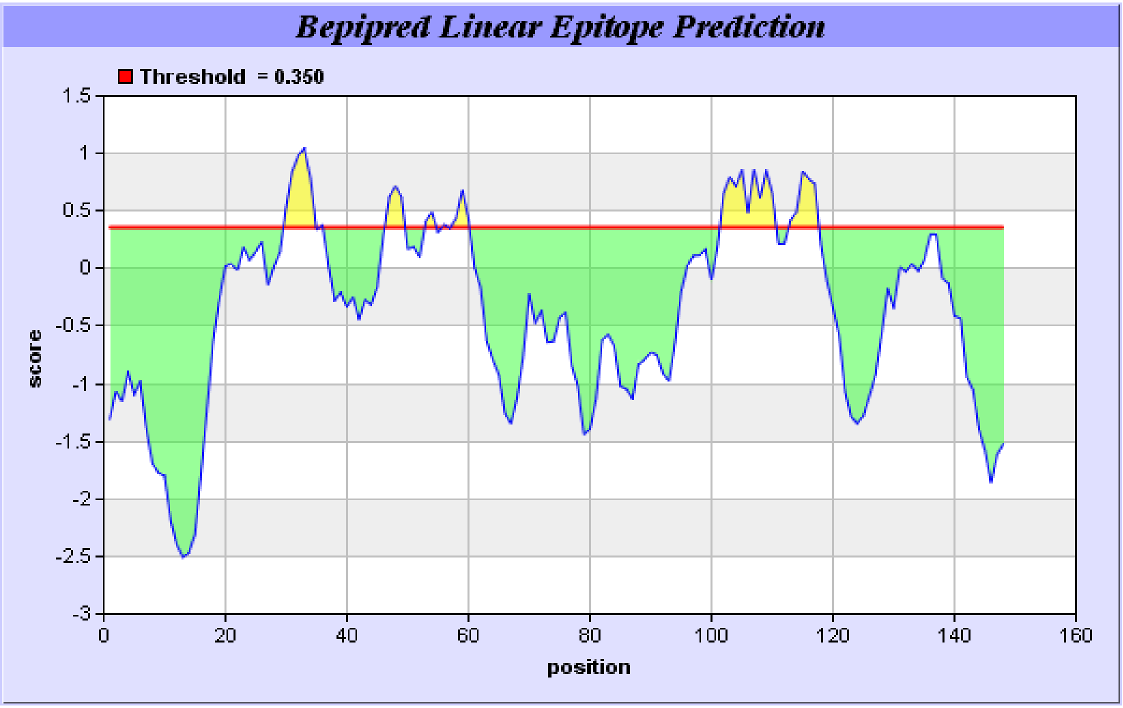
Reference: Reference: Jens Erik Pontoppidan Larsen, Ole Lund and Morten Nielsen. Improved method for predicting linear B-cell epitopes. Immunome Res. 2006; 2: 2.

MGRFIFVSFGLLVVFLSLSGTEAGVCCPLGWSGYDQNCYKAFEE
LMNWADAEKFCTQQHKGSHLVSLHNIAEADFVVKKIVSVLKDGVIWMGLNDV
WNECNW GWTDGAQLDYKAWNVESNCFIFKTAENHWSRTDCSGTHSFVCKSPA

Daboia russelli siamensis(C-Type L-3, Q4PRD0), 148aa(50% identity with over 20 different fractions[4]).

(ref. 5, 6)

No.	Start Position	End Position	Peptide	Peptide Length
1	30	34	GWSAY	5
2	36	36	Q	1
3	47	49	NWA	3
4	53	54	KF	2
5	56	56	T	1
6	58	60	QHK	3
7	102	110	WGWTDG AKL	9
8	113	117	KAWNE	5

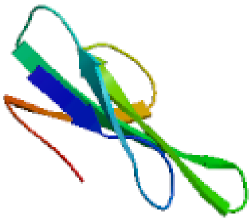


Reference: Reference: Jens Erik Pontoppidan Larsen, Ole Lund and Morten Nielsen. Improved method for predicting linear B-cell epitopes. Immunome Res. 2006; 2: 2.

MGRFISVSFGLLVVFLSLSGTEAAFCCPSGWSAYDQNCYKVFTE
EMNWADAEKFCTEQHKGSHLLSLHNIAEADFVLKKTLAMLKDGVIWMGLNDV
WNECNW
GWTDGAKLDYKAWNEGTNCFVFKIAKNHWSHMDCSSTHNFVCKFRV

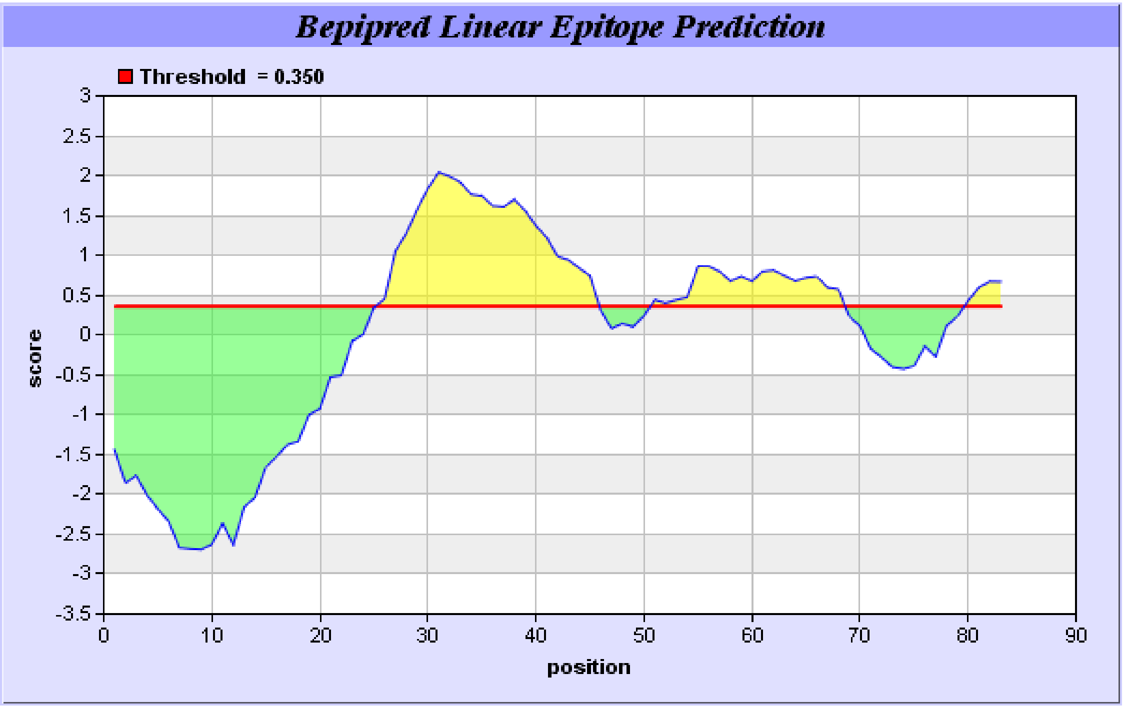
Three-Finger Alpha Neurotoxin Amino Acid Comparisons

Naja naja(alpha neurotoxin, Q9PTT0), 83aa(90% identity with Naja atra Cobratoxin, Naja sputatrix alpha neuro 1,2,3,4, Naja kaouthia short neuro 1, Cobratoxin 2 precursor, Naja mossambica short neuro 1[4]).



(ref. 5, 6)

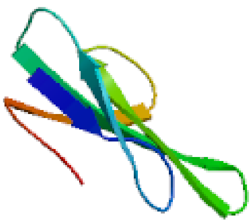
rt Position	End Position	Peptide	Peptide Length	
1	26	45	NQQSSQTPTTTGCSGGETNC	20
2	51	68	RDHRGYRTERGCGCPSVK	18



Reference: Jens Erik Pontoppidan Larsen, Ole Lund and Morten Nielsen. Improved method for predicting linear B-cell epitopes. Immunome Res. 2006; 2: 2.

METLLLTLLVVTIVCLDLGYTLECHNQQSSQTPTTTGCSGGETN
CYKKRWRDHRGYRTERGCGCPSVKNGIEINCCTTDRCNN

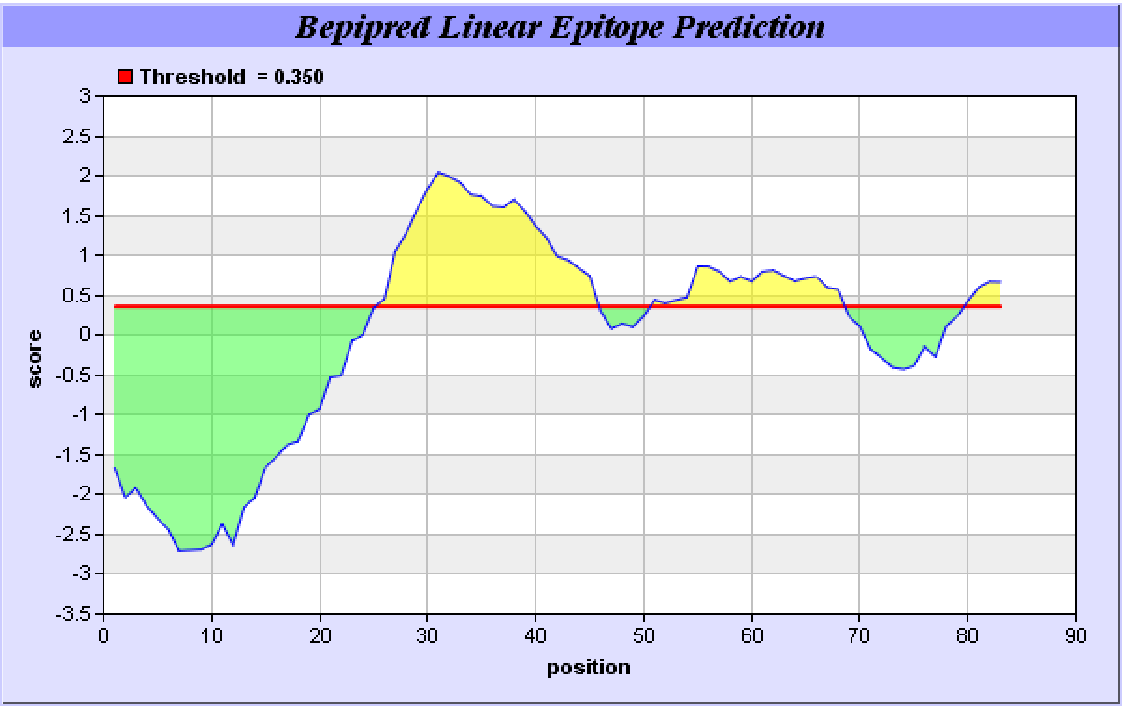
Naja atra(alpha neurotoxin, P60770), 83aa(100% identity with Naja kaouthia and siamensis Cobratoxin, and 90% with 10 other Naja fractions[4]).



(ref. 5, 6)

No.	Start Position	End Position	Peptide	Peptide Length

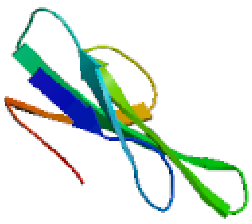
1	26	45	NQQSSQTPTTTGCSGG ETNC	20
2	51	68	RDHRGYRTERGCGCPS VK	18



Reference: Jens Erik Pontoppidan Larsen, Ole Lund and Morten Nielsen. Improved method for predicting linear B-cell epitopes. Immunome Res. 2006; 2: 2.

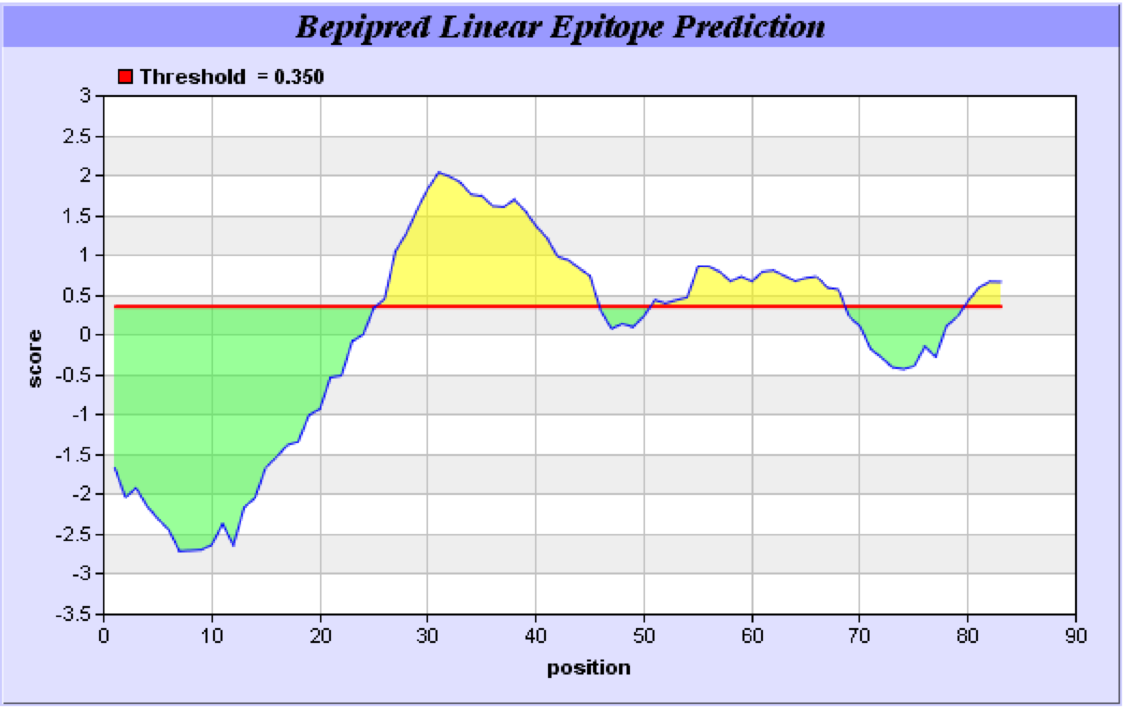
MKTLLLTLLVVTIVCLDLGYTLECHNQQSSQTPTTTGCSGGETN
CYKKRWRDHRGYRTERGCGCPSVKNGIEINCCTTDRCNN

Naja kaouthia(alpha neurotoxin, P60771), 83aa(100% identity with Naja atra, and 90% with 10 other Naja neuro fractions, above[4]).



(ref. 5, 6)

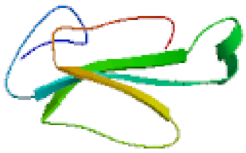
No.	Start Position	End Position	Peptide	Peptide Length
1	26	45	NQQSSQTPTTTGCSGG ETNC	20
2	51	68	RDHRGYRTERGCGCPS VK	18



Reference: Jens Erik Pontoppidan Larsen, Ole Lund and Morten Nielsen. Improved method for predicting linear B-cell epitopes. Immunome Res. 2006; 2: 2.

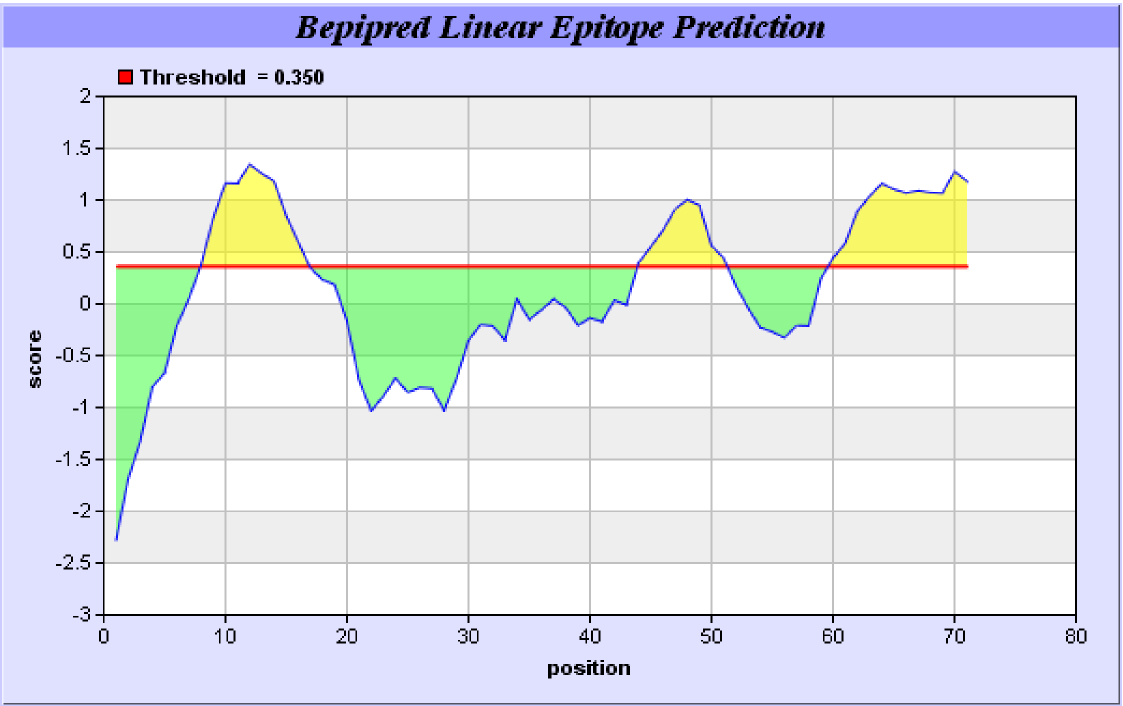
MKTLLLTLLVVTIVCLDLGYTLECHNQQSSQTPTTTGCSGGETN
CYKKRWRDHRGYRTERGCGCPSVKNGIEINCCTTDRCNN

Naja nivea(alpha long neurotoxin 1, P01390), 71aa(50% identity with over 24 other fractions[4]).



(ref 5, 6)

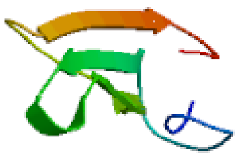
No.	Start Position	End Position	Peptide	Peptide Length
1	8	16	DVTSQACPD	9
2	44	51	TCPKVKPG	8



Reference: Jens Erik Pontoppidan Larsen, Ole Lund and Morten Nielsen. Improved method for predicting linear B-cell epitopes. Immunome Res. 2006; 2: 2.

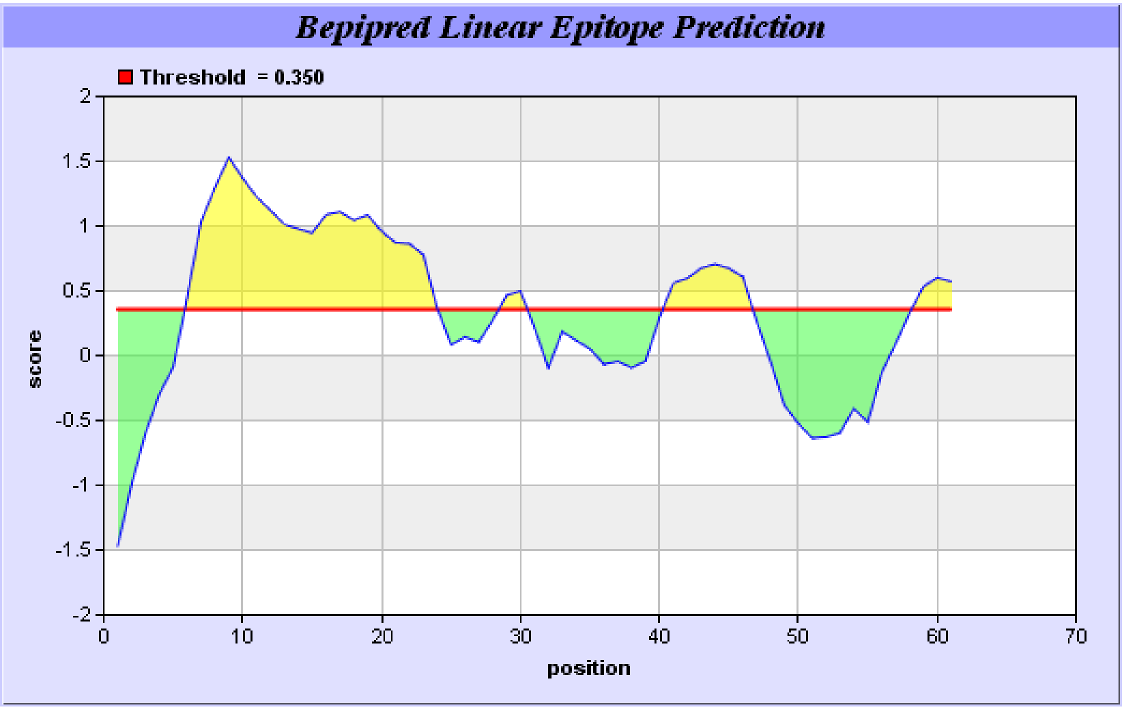
IRCFITPDVTSQACPDGHVCYTKMWCDNFCGMRGKRVDLGCAATCPKVKPGVN
IKCCSRD
NCNPFPTRKRS

Naja nivea(alpha short neurotoxin II, P01423), 61aa(90% identity with short neurotoxin II from Naja haje haje and Naja haje annulifera[4]).



(ref. 5, 6)

No.	Start Position	End Position	Peptide	Peptide Length
1	6	24	QQSSQRPTIKTCPGETNCY	19
2	29	30	RD	2
3	41	46	GCPSVK	6



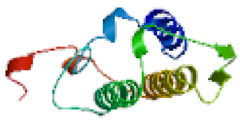
Reference: Jens Erik Pontoppidan Larsen, Ole Lund and Morten Nielsen. Improved method for predicting linear B-cell epitopes. Immunome Res. 2006; 2: 2.

MICHNQQSSQPPTIKTCPGETNCYKKRWRDHRGTIIERGCGCPSVKKGVGIYCCK

TNKCN
R

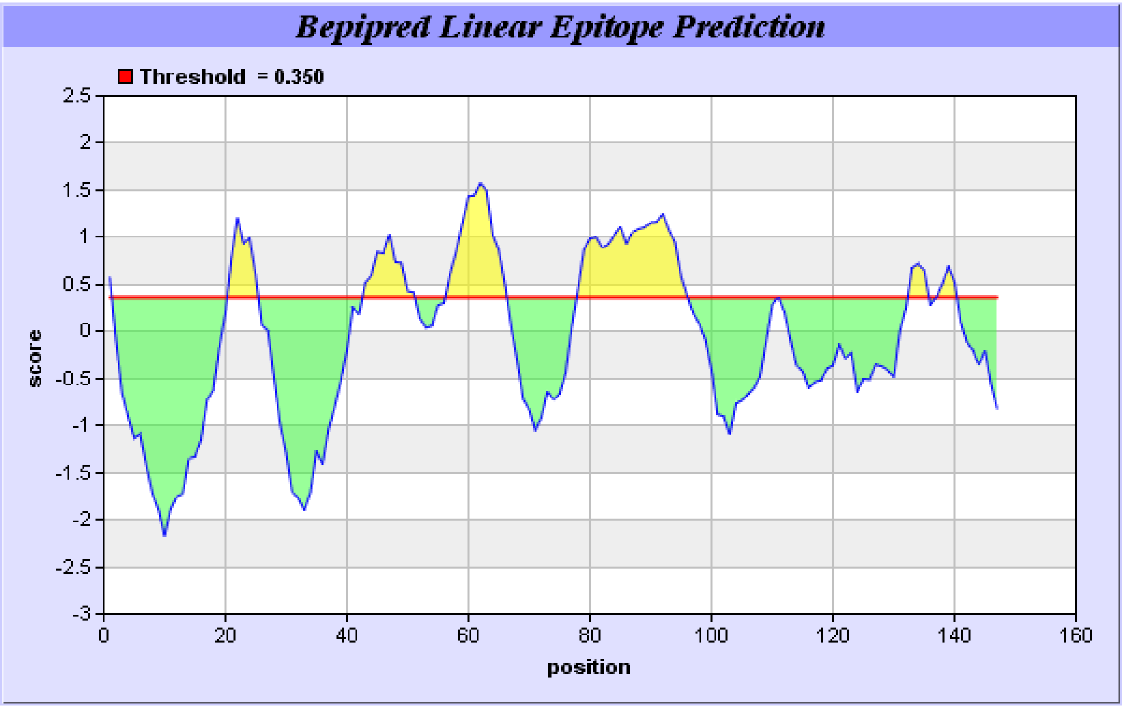
Presynaptic Beta Neurotoxin Amino Acid Comparisons

Bungarus multicinctus(beta PLA2 bungarotoxin A1 chain, P00617), 147aa



(ref. 5, 6)

	Start Position	End Position	Peptide	Peptide Length
1	1	1	M	1
2	21	25	NIPPH	5
3	43	51	EKTWGEYAD	9
4	57	66	GAGGSGRPID	10
5	78	96	CYGDAEKKHKCNPKTQSYS	19
6	111	111	G	1
7	133	135	YIE	3
8	137	140	HKNI	4

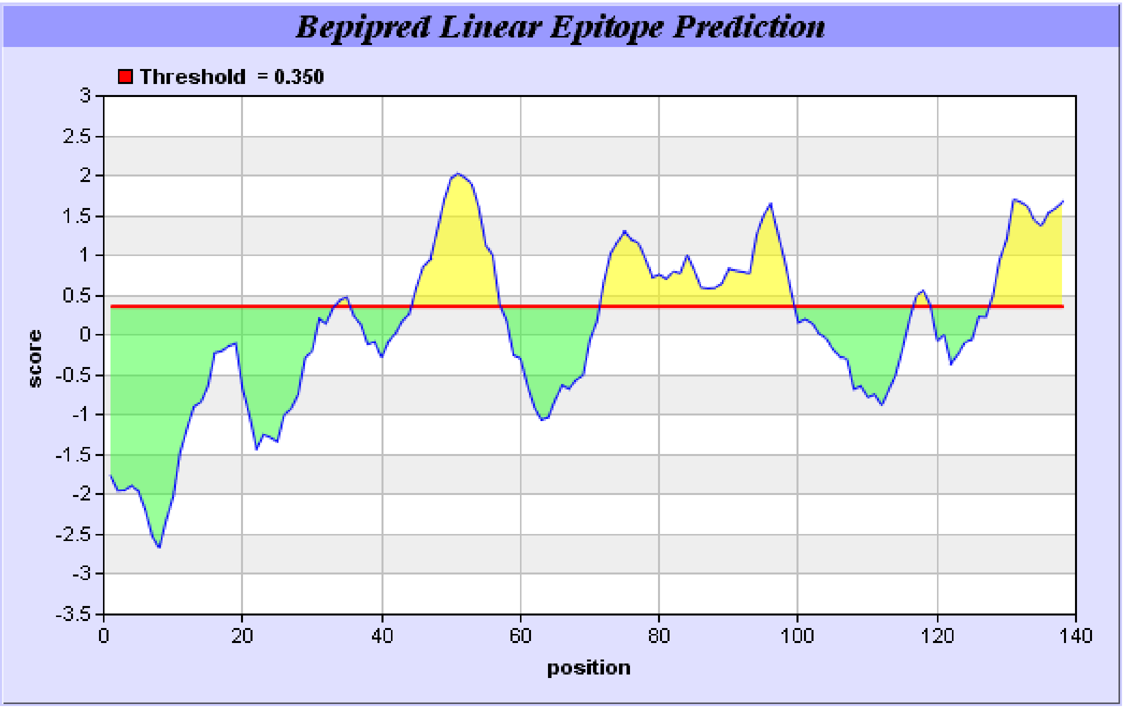


Reference: Jens Erik Pontoppidan Larsen, Ole Lund and Morten Nielsen. Improved method for predicting linear B-cell epitopes. Immunome Res. 2006; 2: 2.

AVCVSLLGAANIPPHPLNLINFMEMIRYTIPCEKTWGEYADYGCY FT CGAGGSGRPIDALDRCCYVHDNCYGDAEKKHKCNPKTQSYSYKLTKRTIICYGA AGTCG FT RIVCDCDRTAALCFGNSEYIEGHKNIDTARFCQ

Crotalus durissus terrific us(beta PLA2 Crotoxin, P08878), 138aa.(90% identity with Mojave rattlesnake Mtx-a[4]).

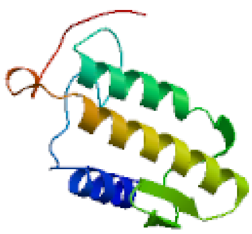
No.	Start Position	End Position	Peptide	Peptide Length
1	34	35	IS	2
2	45	57	GAGGQGWPQDASD	13
3	72	99	GCDPTTDVYTYRQEDGEIVC GEDDPCGT	28
4	117	119	DTY	3



Reference: Jens Erik Pontoppidan Larsen, Ole Lund and Morten Nielsen. Improved method for predicting linear B-cell epitopes. Immunome Res. 2006; 2: 2.

MRALWIVAVLLVGVEGSLVEFETLMMKIAGRSGISYYSSYGCYCG FT
AGGQGWPQDASDRCCFEHDCCYAKLTGCDPTTDVYTYRQEDGEIVCGEDDPCG
TQICEC FT DKAAAICFRNSMDTYDYKYLQFSPENCQGESQPC

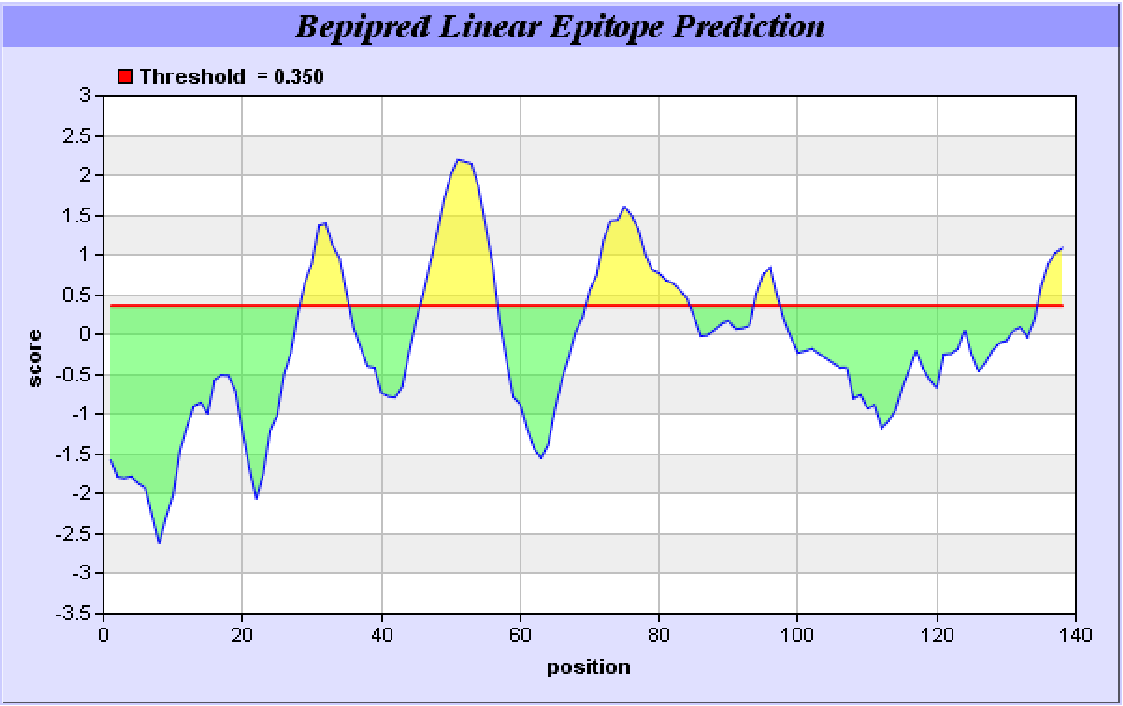
Vipera ammodytes(Ammodytoxin, P00626), 138aa(4)



(ref. 5, 6)

No.	Start Position	End Position	Peptide	Peptide Length
1	29	35	TGKNPLT	7

2	46	56	VGGKGTPKDAT	11
3	70	84	LPDCSPKTDRYKYHR	15
4	94	97	GTSC	4



Reference: Jens Erik Pontoppidan Larsen, Ole Lund and Morten Nielsen. Improved method for predicting linear B-cell epitopes. Immunome Res. 2006; 2: 2.

MRTLWIVAVCLIGVEGSLLEFGMMILGETGKNPLTSYSFYGCYC GVGGKGTPKDATDRCCFVHDCCYGNLPDCSPKTDRYKYHRENGAIVCGKGTSC ENRIC ECDRAAAICFRKNLKTYNYIYRNYPDFLCKKESEKC

Conclusion

There are many very positive features that can be drawn from these amino acid comparisons. The complete DNA sequences are already done for all these venom fractions, which makes it easier to develop a DNA-based vaccine for human use.

The structural similarity with these fractions means that IgG raised antibodies will cross with not just different species, but different genera, in different continents. There are many 50% amino acid similarities, but the 100% and 90% fractions show that cross-reactivity will happen. The alpha neurotoxins and CTL toxins are a very good example of how similar that group is. Truly, a partial multi-continental ophidian DNA -based vaccine.

My personal opinion would be having three continental DNA vaccines for humans(partial of course at this point). South America would have the DNA-jararhagin vaccine, Africa would have the DNA-ecarin/neuro-alpha(Naja), and Asia would have the DNA-ecarin/neuro-alpha(Naja)/RVV-X(Daboia)/bungarotoxin(Bungarus).

Obviously more fractions could be added, but I feel that's a good initial mix for partial coverage. At first though, I think just zeroing in on one fraction would be the key to see if IgG acceptable titers can be achieved.

References

1) Chippaux, J.P. http://www.who.int/bloodproducts/publications/en/bulletin_1998_76(5)_515_524.pdf

2) Harrison , R. Development of Venom Toxin Specific Antibodies.Vaccine, V22, No. 13-14, pp. 1648-55, 2004.

3) Reptile Database. http://www.tigr.org/reptiles/search.php.

4) Swiss-Prot. http://expasy.org/cgi-bin/sprot-search-ful

5) Jurge Kopp and Torsten Schwede(2004). The SWISS-MODEL Repository of annotated 3D protein structure homology models-nucleic acids res., 32, D230-D234.

6) Schwede, T., Kopp, J., Guex, N., and Peitsch, M.C.(2003). SWISS-MODEL: An automated protein homology modeling server. Nucleic acids res., 31, 3381-3385.