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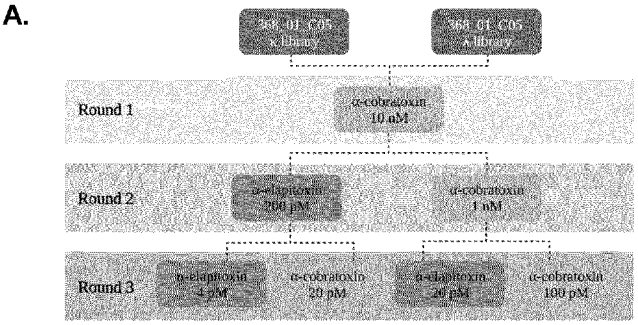

Fig. 1

(57) Abstract: In the field of recombinant antivenoms, a major hurdle to the development of antigen-binding proteins enabling sufficient toxin neutralization is the identification of antigen-binding proteins with both high affinity and broad cross-reactivity against venom toxins. Traditional antibody development approaches used failed to isolate antigen-binding proteins against venom toxins with such properties. The present disclosure describes the development of high-affinity human antibodies that show broadly-neutralizing effects against neurotoxic elapid snake venom in vitro and in vivo. The invention relates to antigen-binding proteins capable of binding to, blocking, or neutralizing two or more different α-neurotoxins. The disclosure also provides pharmaceutical compositions, kit of parts and kits for the treatment and diagnosis of snake envenomation.

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Neutralisation of α-neurotoxins using human recombinant IgG antibodies

Technical field

The present invention relates to antibodies which bind, block and/or neutralize α-neurotoxins from snake venom.

Background

Each year, snakebite envenoming exacts a high death toll and leaves hundreds of thousands of other victims maimed for life. Antivenoms based on polyclonal antibodies isolated from plasma of immunized animals are currently the only specific treatment option against severe envenomings. These medicines are essential and life-saving, and will remain a cornerstone in snakebite therapy for years to come.

Advances in biotechnology and antibody discovery have enabled the development of recombinant antivenoms based on an oligoclonal mixture of human immunoglobulins. These human recombinant oligoclonal antivenoms are different from polyclonal antibodies-based therapies. They are well characterized, human of origin, can be manufactured without the use of animals, and have significantly reduced risks of causing adverse reactions in humans.

However, it is essential that recombinant antivenoms can be designed to be polyvalent, *i.e.*, target multiple different snake species, without dramatically increasing dose or cost of manufacture.

The design of such recombinant antivenoms remains a challenge. Within the field of recombinant monoclonal antibodies, no studies have been published showing discovery of a broadly neutralizing antibody against α-neurotoxins.

Summary

The invention is defined in the attached claims.

Traditional approaches for envenomation treatment involve the use of polyclonal and/or oligoclonal immunoglobulins, initially from animal sources and more recently of human recombinant origin.

An important limitation of these treatments is still however the lack of broadly neutralizing options, which could improve the efficacy of the antivenoms and reduce side-effects.

Monoclonal antibodies targeting snake venom toxins can be developed using various platforms, such as phage display technology (**Ref. 2**), an *in vitro* methodology that can be used to actively select for antibodies with high-affinity and cross-reactivity (**Refs. 3 and 4**). In addition, use of human antibody libraries in combination with phage display technology allows for the discovery of fully human antibodies, which frequently have high treatment tolerability in patients. Phage display technology could be particularly valuable for discovering monoclonal antibodies against highly potent toxins with low immunogenicity that fail to elicit a strong antibody response in animals used for immunization, such as low molecular mass neurotoxins and cytotoxins of the three-finger toxin (3FTx) family, which are abundant in Elapidae venoms, such as cobra and mamba venom (**Refs. 5 and 6**).

However, antibodies derived directly from naïve phage display libraries often lack sufficiently high affinity to enable toxin neutralization (**Ref. 4**).

The invention however presents high affinity human antibodies. Thus, by developing high-throughput pipelines for the generation of high affinity broadly-neutralizing antibodies, the present inventors were capable of developing broadly-neutralizing, high affinity human monoclonal antibodies. In particular, the invention provides a broadly-neutralizing human monoclonal antibody that is capable of preventing lethality induced by *N. kaouthia* whole venom at an unprecedented low molar ratio of one antibody per toxin, and which also prolongs survival of mice injected with *Dendroaspis polylepis* or *Ophiophagus hannah* whole venom. In addition, the antibody is capable of binding toxins from several other snakes as outlined below.

The antibodies of the invention have not been developed by site-directed or random mutagenesis of antibody paratopes. Instead, a phage display library was generated by paring a heavy or light chain from a specific antibody with a naïve repertoire of the opposite chain and performing a new selection campaign.
For the first time, the inventors show that this technology could be used to generate antibodies that possess high affinity while simultaneously having a broad neutralization capacity, i.e., able to neutralize several related toxins from the venoms of different snake species.

The human monoclonal antibody, 368_01_C05, against α-cobratoxin (P01391), a potent neurotoxin from the monocled cobra, *Naja kaouthia* has been discovered using a naïve human scFv-based phage display library (**Ref. 7**). Notably, the 368_01_C05 antibody could prolong the survival of mice injected with lethal doses of α-cobratoxin, although it failed to prevent lethality (**Ref. 7**).

The present invention provides an antibody, which is significantly superior to 368_01_C05. The inventors have constructed light-chain-shuffled antibody libraries based on 368_01_C05, and used a phage display-based cross-panning campaign to simultaneously improve the affinity and expand the neutralizing capacity of the antibody against α-neurotoxins from venoms of several snake species. Cross-panning was carried out between α-cobratoxin (**Ref. 8**) and α-elapitoxin (**Ref. 9**), a neurotoxin from the venom of the black mamba, *Dendroaspis polylepis* (**Ref. 6**). These two α-neurotoxins share 70% sequence identify and both cause neuromuscular blockade by binding to the nicotinic acetylcholine receptor (nAChR) in muscle cells. The chain-shuffled scFv library was “cross-panned” using these two toxins as antigens under stringent conditions to discover antibodies with improved affinity and cross-reactivity in comparison to the parent antibody.

α-neurotoxins are a group of toxins present in a large part of the African and Asian elapid snakes, and as the toxins interfere with neuromuscular transmission causing paralysis and suffocation of humans envenomed, they are extremely important to neutralize. Many α-neurotoxins have sequence similarities, and the antibodies of the present invention are capable of binding to several α-neurotoxins.

The present invention provides amongst others six recombinant human monoclonal IgG antibodies that bind α-neurotoxins from African and Indian elapid snakes.

These six IgGs are referred to as 2551_01_A12, 2554_01_D11, 2558_02_G09, 2551_01_B11, 2555_01_A01, and 2555_01_A05 herein. The IgGs are capable of both binding, blocking, and *in vitro* and *in vivo* neutralization of different purified toxins or whole snake venoms. A preferred antibody of the invention is 2554_01_D11, which not only has improved affinity to α-cobratoxin, but also significantly broadened cross-neutralization capacity against other α-neurotoxins from the venoms of elapid snakes from the genera *Dendroaspis, Ophiophagus, Bungarus*, and *Naja*. Another preferred

antibody of the invention is 2555_01_A01 also showing improved affinity to α-cobratoxin and cross neutralization against at least other α-neurotoxins from the venoms of elapid snakes from the genera *Dendroaspis* and *Naja.*

The antibodies of the invention are promising candidates to include in recombinant antivenoms against venoms with a high content of α-neurotoxins (such as black mamba or cobra venoms).

Recombinant antivenom based on the antigen-binding proteins described in the present invention will bring safer, more efficacious, and cheaper treatments for snakebite victims, in particular in Asia and Africa.

A first aspect of the present invention relates to an antigen-binding protein capable of binding to, blocking, or neutralizing two or more different α-neurotoxins.

A second aspect of the invention relates to an antigen-binding protein comprising or consisting of a heavy chain variable (VH) region comprising a heavy chain complementarity-determining region 3 (CDR-H3) comprising or consisting of any of SEQ ID NO: 19, 11, 27, 35, 43 and 51 or a variant thereof, wherein in said variant up to 3 amino acids have been altered , for example wherein 2 or 1 amino acid(s) have been altered .

A third aspect of the present invention relates to a pharmaceutical composition comprising an antigen-binding protein as defined herein, and a pharmaceutically acceptable diluent, carrier and/or excipient.

A fourth aspect of the present invention relates to a kit-of-parts comprising:

a) an antigen-binding protein as described herein; and
b) another agent suitable for the treatment of snake envenomation.

A fifth aspect of the present invention relates to an antigen-binding protein as described herein, a pharmaceutical composition as described herein or a kit-of-parts as described herein for use in a method of treatment of snake envenomation in a subject.

A sixth aspect of the present invention relates to a method of treating snake envenomation which comprises administering to a subject an effective amount of the antigen-binding protein as described herein, the composition as described herein, or the kit-of-parts as described herein.

A seventh aspect of the present invention relates to the use of the antigen-binding protein as described herein, the composition as described herein, or the kit-of-parts as described herein, for the manufacture of a medicament for the treatment of snake envenomation.

An eighth aspect of the invention relates to a kit for detection and/or quantification of snake venom in a sample comprising:

- one or more antigen-binding proteins, such as two antigen-binding proteins, wherein at least one of said antigen-binding proteins is as described herein; and
- means for detection of a complex comprising said one or more antigen-binding proteins bound to snake venom.

A ninth aspect of the invention relates to an antigen-binding protein as described herein for use in the diagnosis of snake envenomation in a subject.

A tenth aspect of the present invention relates to the use of an antigen-binding protein as described herein in an *in vitro* method for detection and/or diagnosis of snake envenomation in a subject.

An eleventh aspect of the present invention relates to a method for detecting snake venom in a sample, said method comprising:

a) providing a sample for analysis;
b) contacting the sample with one or more antigen-binding proteins as defined herein to said sample; and

detecting a complex comprising said one or more antigen-binding proteins bound to a snake antigen.

Description of Drawings

Figure 1 : Cross-panning selection strategy as well as assay and sequence data for selected IgGs. A. Selection strategy illustrating how cross-panning was performed, including antigen concentrations. B. ENC DELFIA showing cross-reactivity of top six affinity matured IgGs (2551_01_A12, 2551_01_B11, 2554_01_D11, 2555_01_A01, 2555_01_A05, and 2558_02_G09) in comparison with parental IgG (368_01_C05) and clone 2552_02_B02 from a previously published study (**Ref. 4**). C. Comparison of CDR-L, CDR-L2, and CDR-L3 sequences for the top six chain-shuffled antibodies and the parental antibody.

Figure 2 : **Affinity measurements using surface plasmon resonance**. Sensograms illustrating affinity measurements of the top six affinity matured antibodies as well as the parent on α-cobratoxin (A.) and α-elapitoxin (B.) immobilized on a CM5 sensor.

Figure 3 : **Size exclusion chromatograms of the snake whole venoms and native mass spectra of toxin:antibody complexes.** Size exclusion chromatograms of IgG 2554_01_D11 and five featured venoms accompanied by native mass spectra of IgG 2554_01_D11 – toxin fraction with asterisk from each SEC run.

Figure 4 : Intact masses and top-down sequence analysis of toxins bound by 2554_01_D11. Mass spectra have been arranged from top to bottom for each species as follows; *O. hannah*, *N. naja*, *N. kaouthia*, *N. melanoleuca* and *D. polylepis*. The spectra on the left-hand side show the charge state distribution for the toxins ejected from the antibody complex by applying a high cone voltage, where the masses of the identified toxins are given in Daltons. The top-down sequence spectra for the most prominent charge state of each toxin are shown in the right-hand side. The difference in m/z are indicated by the width between the dotted lines on top and match the specific amino acid or peptide. The full amino acid sequence for the proposed identity of the toxins is given below each spectrum, with the matching peptides found during the top-down analysis underlined. Cysteines in the sequence appear as light grey.

Figure 5 : Alignment and epitope identification of all investigated long chain α-neurotoxins, i.e. α-cobratoxin (P01391/1CTX) from *N. kaouthia*, α-elapitoxin (P01396/AF-P01396) from *D. polylepis*, α-bungarotoxin (P60615/1HC9) from *B. multicinctus*, long neurotoxin 2 (A8N285/AF-A8N285) from *O. hannah*, long neurotoxin (P0DQQ2), long neurotoxin 4 from *N. naja* (P25672/AF-P25672), and

long neurotoxin 2 (P01388/AF-P01388) from *N. melanoleuca*. A. Sequence alignment using Clustal Omega with black boxes indicating residues involved in binding to the nicotinic acetylcholine receptor or bound by antivenom antibodies. B. Structural alignment in ChimeraX. C) Amino acid residues on α-cobratoxin known to be involved in binding to its native target, i.e., the nicotinic acetylcholine receptor (**Ref. 12**) (black). D) Amino acid residues in α-elapitoxin suggested to be bound by antivenom antibodies based on high-density peptide microarray analysis (**Ref. 11**) (black). E) Amino acid residues in long neurotoxin 2 from *N. melanoleuca* suggested to be bound by antivenom antibodies based on high-density peptide microarray analysis (**Ref. 11**) (black).

Figure 6 : Electrophysiological determination of the *in vitro* cross-neutralizing potential of 2551_01_A12, 2554_01_D11, and 368_01_C05. Automated patch-clamp experiments were performed to determine the ability of the antibodies to prevent the current-inhibiting effect that α-neurotoxins exert on the nAChR. A. Concentration-response curves illustrating how increasing concentrations of the three antibodies prevent nAChR blocking by α-cobratoxin. B. Single concentration plot outlining the cross-neutralizing potential of the antibodies against α-cobratoxin from *N. kaouthia*, α-elapitoxin from *D. polylepis*, α-bungarotoxin from *B. multicinctus*, and Nm8 from *N. melanoleuca*. In addition, a negative control Nm3, a fraction from *N. melanoleuca* venom containing a short α-neurotoxin, was included. The toxin to antibody molar ratios used were 1:22 for α-cobratoxin, 1:40 for α-elapitoxin, 1:5 for α-bungarotoxin, 1:2.3 for Nm8, and 1:3.2 for Nm3.

Figure 7 : Kaplan-Meier survival curves for the antibody 2554_01_D11. A., B., and C. Mixtures containing 2 LD50s of venom of either *N. kaouthia*, *O. hannah*, or *D. polylepis* were preincubated with the antibody, 2554_01_D11, at various toxin:antibody ratios and then administered intravenously (i.v). to groups of four mice. Controls included mice receiving venom alone or venom incubated with either an irrelevant isotype antibody control or a commercial horse-derived antivenom. Signs of toxicity were observed, and deaths were recorded for a maximum period of 48 hours. D. 2 LD50s *N. kaouthia* venom was administered subcutaneously (s.c.) following i.v. administration of IgG 2554_01_D11 either immediately following venom administration or 10 minutes after venom administration. As a control, mice were administered venom

s.c. and PBS i.v. Signs of toxicity were observed, and deaths were recorded for 24 hours.

Figure 8 : Kaplan-Meier survival curves for the antibody 2555_01_A01.
A mixture comprising 2 median lethal doses (LD50s) of α-cbtx (α-cobratoxin) was preincubated with the antibody, 2555_01_A01, at 1:1 and 1:2 toxin:antibody ratios and then administered intravenously (i.v). to groups of four mice. Controls included mice receiving α-cbtx alone (α-cbtx only), or α-cbtx incubated with an isotype control IgG. Signs of toxicity were observed, and deaths were recorded for a maximum period of 48 hours.

Detailed description

Definitions

As used herein, the singular forms “a”, “an” and “the” include plural referents unless the context clearly states otherwise. Thus, for example, reference to “an antigen-binding protein” includes a plurality of such antigen-binding proteins.

As used herein, the term “antigen-binding protein” includes antibodies, antibody fragments and other antigen-binding protein constructs. The term encompasses intact antibodies that comprise at least two full-length heavy chains and two full-length light chains, as well as derivatives, variants, fragments, and mutations thereof, examples of which include Fab, Fab', $F(ab')_2$, and Fv fragments. An antigen-binding protein also includes domain antibodies such as nanobodies, camelid single domain antibodies, heavy chain variable region fragment (V_H) and single-chain antibodies.

The terms “long-chain-α-neurotoxins”, “long-chain neurotoxin”, “long neurotoxin” are used herein as equivalent terms.

As used herein the term “corresponding to” in terms of amino acids, means that following alignment between a polypeptide and a reference polypeptide, an amino acid corresponds to position X of the reference sequence if it aligns to the same position.

As used herein, the term “variant” refers to either a naturally occurring variation of a given peptide or a recombinantly prepared variation of a given peptide or protein in

which one or more amino acid residues have been modified by amino acid substitution, addition, or deletion. The term “variant” may also define either a naturally occurring genetic mutant of a DNA sequence or its encoded RNA or protein product, or a recombinantly prepared variation of a DNA sequence or its encoded RNA or protein product.

As used herein, the term “altered” in terms of amino acids encompasses “deleted”, “inserted” or “substituted with another amino acid”. Preferably, “altered” as used herein refers to “substituted for another amino acid”

As used herein, "K_d" refers to the equilibrium dissociation constant for a ligand-receptor complex. The K_d value is expressed in Molar units (M) and is obtained by dividing the dissociation rate (k_{off}) by the association rate (k_{on}). The association rate, the dissociation rate and the equilibrium dissociation constant are used to represent the binding affinity of an antibody to an antigen

As used herein the term “developability” refers to the suitability of the antigen-binding protein clones for future antibody development. It can be investigated by characterizing biophysical properties that are indicative of how well the antibodies can be developed and manufactured in large scale without aggregating, precipitating, denaturing, or having suboptimal pharmacokinetics *in vivo*. For example an Affinity-Capture Self-Interaction Nanoparticle Spectroscopy (AC-SINS) assay as described herein and in (**Ref 10)** can be used to evaluate self-aggregation based on the plasmon shift observed**.** The skilled person will appreciate that the purity of the antigen-binding proteins and their non-specific interactions can be assayed for example by chromatography techniques, such as size exclusion chromatography (SEC), affinity chromatography techniques, such as protein A or protein G affinity purification, Ion-exchange chromatography techniques, or Immobilized metal chelate chromatography (IMAC).

Antigen-binding protein capable of binding to, blocking, or neutralizing two or more different α-neurotoxins

The present invention relates to an antigen-binding protein capable of binding to, blocking, or neutralizing two or more different α-neurotoxins.

The term “blocking” as used herein refers to the binding of the antigen-binding protein to the antigen and interference with the antigen function, for example preventing the antigen from binding to other antigen-binding proteins and/or other target ligands of the antigen.

Numerous α-neurotoxins exist, because different snakes produce different α-neurotoxins, some of which are highly similar. Interestingly, the antigen-binding protein of the invention are capable of binding several α-neurotoxins. In some embodiments, the antigen-binding protein is capable of binding to, blocking or neutralizing at least 3, such as at least 4, for example at least 5, such as at least 6, for example at least 7, such as at least 10, for example at least 20, such as at least 50 different α-neurotoxins. Whereas there in principle is no upper limit to the number of different α-neurotoxins the antigen-binding protein can bind, frequently the antigen-binding protein is not capable of binding more than 200 different α-neurotoxins. Said α-neurotoxins may preferably be any of the α-neurotoxins described herein below in the section “α-neurotoxins from snake venom”.

The antigen-binding proteins of the present invention preferably have high affinity for the antigen, such as α-neurotoxins. As used herein “binding to” refers to the formation of a non-covalent association between the antigen-binding protein and the antigen, although such binding is not necessarily reversible. The K_d mentioned hereinabove can be used to measure the affinity of the binding, wherein a lower relative K_d value indicates a higher affinity for the antigen.

In embodiments, the antigen-binding protein binds to two or more different α-neurotoxins with a K_d of at the most 8.5 nM, such as at the most 9 nM, for instance at the most 9.5nM, such as at the most 10 nM, for instance at the most 12.5nM, such as at the most 15 nM, for instance at the most 20nM, such as at the most 25 nM, for instance at the most 50nM, such as at the most 75 nM, for instance at the most 85nM.

In some embodiments the antigen-binding protein binds to two or more different α-neurotoxins with a K_d of at the most 10 nM.

In some embodiments, the antigen-binding protein binds to two or more different α-neurotoxins with a K_d of at the most 5 nM.

The person skilled in the art will appreciate that antigen-binding protein affinity can be measured for instance using Surface Plasmon Resonance (SPR), such as described for example herein in Example 1 or in **Ref 7.**

The term “neutralizing” as used herein refers to an antigen-binding proteins which reduces or abolishes the biological activity of an antigen, such as a snake venom antigen, for instance an α-neurotoxin.

In some embodiments the neutralization is obtained at a α-neurotoxin-to-antigen-binding protein molar ratio of no more than 1:1, such as no more than 1:1.5, for instance no more than 1:2, such as no more than 1:2.5, for instance no more than 1:3, such as no more than 1:3.5, for instance no more than 1:4, such as no more than 1:5.

"IC50" as used herein, refers to the concentration of an agent which produces 50% of the maximal inhibitory response possible for that agent or, alternatively, to the concentration of an agent which produces 50% displacement of ligand binding to the receptor.

In some embodiments of the present invention, the IC50 value of neutralization of the α-neurotoxin by the antigen-binding protein is no more than 6 nM, such as no more than 5.5 nM, for instance no more than 5nM, such as no more than 4.5 nM, for instance no more than 4 nM, for instance no more than 3.5 nM, such as no more than 3 nM, for instance no more than 2.5 nM, such as no more than 2 nM, for instance no more than 1.5 nM, such as no more than 1 nM.

The person skilled in the art will appreciate that the antigen-binding proteins recognize epitopes of the antigen. For example, an epitope can comprise or consist of a portion of a peptide (e.g., an α-neurotoxin). An epitope can be a linear epitope, sequential epitope, or a conformational epitope.

The epitopes of the α-neurotoxins may be conserved even though the overall sequence identity may be relatively low. Thus, as shown in the sequence alignments in Example 2 different α-neurotoxins have an overall sequence identity as low as 31%, however, antibodies of the invention can bind several of these α-neurotoxins.

A total of 29 positions in the toxin sequences contain identical amino acids across seven α-neurotoxins (Example 2), including most of the residues previously identified as playing a significant role in the binding between α-cobratoxin/α-bungarotoxin and the nAChR (**Ref. 12**). Specifically, these amino acid residues include Trp25, Cys26, Asp27, Ala28, Phe29, Cys30, Arg33, Lys35, and Arg36/Val39 (α-cobratoxin/α-bungarotoxin) on loop II and Phe65/Val39 (α-cobratoxin/α-bungarotoxin) on the C-terminus, where a single mutation of one of these residues has been shown to cause a more than five-fold decrease in affinity to the nAChR. This emphasizes the potential importance of Trp25, Cys26, Asp27, Ala28, Phe29, Cys30, and Arg36 for the ability of antibodies to recognize this toxin. Together, this presents a plausible explanation for the broad cross-reactivity observed for example for 2554_01_D11 and indicates the importance of epitope similarity in the pursuit of cross-reactive antibodies. Accordingly, it is preferred that the antigen-binding protein of the invention binds a epitope comprising one or more of amino acid residues Trp25, Cys26, Asp27, Ala28, Phe29, Cys30, Arg33 and/or Lys35 of α-cobratoxin and/or Arg36/Val39 (α-cobratoxin/α-bungarotoxin) and/or Phe65/Val39 (α-cobratoxin/α-bungarotoxin).

In some embodiments, the antigen-binding protein is capable of binding to an epitope contained between residues corresponding to amino acids 24 to 60 of the toxin protein sequences as defined in any one of SEQ:IDs No 56, 57, 58, 59 and 60 or variant(s) thereof having at least 70% identity, such as at least 75% identity, for example at least 80% identity, such as at least 85% identity, for example at least 90% identity, such as at least 95% identity thereto, preferably wherein the antigen-binding protein is capable to bind to an epitope contained between residues corresponding to amino acids 41 to 60 of the toxin protein sequences as defined in any one of SEQ:IDs No 56, 57, 58, 59 and 60 or variant(s) thereof having at least 70% identity, such as at least 75% identity, for example at least 80% identity, such as at least 85% identity, for example at least 90% identity, such as at least 95% identity thereto.

In some embodiments, the antigen-binding protein is capable of binding an epitope having the sequence of SEQ ID NO: 69: CAATCPXVKXGVXIXCCST,
wherein X may be any amino acid.

The skilled person will appreciate that it is preferred that the antigen-binding proteins have a low wavelength shift as measured by AC-SINS, as this measurement is indicative of the tendency to self-aggregation.

In some embodiments of the present invention, the AC-SINS shift of the antigen-binding protein is of no more than 11nm, for example no more than 15nm, such as no more than 20nm, for example no more than 25nm, such as no more than 30nm.

α-neurotoxins from snake venom

The person skilled in the art will appreciate that α-neurotoxins are a group of toxins present in a large part of the African and Asian elapid snakes venoms.

In some embodiments of the present invention, the antigen-binding protein is capable of binding to, blocking or neutralizing two or more different long-chain α-neurotoxins, for example at least 3, such as at least 4, for example at least 5, such as at least 6, for example at least 7 different long-chain α-neurotoxins, wherein said antigen-binding protein binds to two or more different long-chain α-neurotoxins with a Kd of at the most 10nM.

In some embodiments of the present invention, the antigen-binding protein of the invention is capable of binding at least two α-neurotoxins, which are long-chain-α-neurotoxins. Thus, the antigen-binding protein may be capable of binding to, blocking or neutralizing at least 3, such as at least 4, for example at least 5, such as at least 6, for example at least 7, such as at least 10, for example at least 20, such as at least 50 different long-chain-α-neurotoxins.

In other embodiments, the antigen-binding protein is capable of binding to, blocking or neutralizing at least two α-neurotoxins selected from the group consisting of α-elapitoxins and α-bungarotoxins. One of said α-elapitoxins may for example be α-elapitoxin-Dppd2.

The antigen-binding protein may in particular also be capable of binding to, blocking or neutralizing α-cobratoxins.

The skilled person will appreciate that whole snake venom can be fractionated using for example size exclusion chromatography (SEC) and that different mixtures of α-neurotoxins have been isolated from different snake species.

In some embodiments of the present invention, the antigen-binding protein of the invention are capable of binding at least two, such as at least 3, for example at least 4, such as at least 5, for example at least 6 α-neurotoxins selected from the group consisting of long neurotoxin 2 (A8N285) from *O. hannah*, α-cobratoxin (P01391) from *N. kaouthia*, long neurotoxin 2 (P01388) and long neurotoxin (P0DQQ2) from *N. melanoleuca*, long neurotoxin 4 (P25672) from *N. naja*, and α-elapitoxin (P01396) from *D. polylepis*.

In some embodiments of the present invention, the antigen-binding protein of the invention are capable of binding at least 3 different long chain α-neurotoxins selected from the group consisting of α-elapitoxin, α-cobratoxin and Nm8.

In some embodiments of the present invention, the antigen-binding protein of the invention are capable of binding at least 3 long chain α-neurotoxins selected from the group consisting of long neurotoxin (P0DQQ2) from *N. melanoleuca*, α-cobratoxin (P01391) from *N. kaouthia* and α-elapitoxin (P01396) from *D. polylepis*. Long neurotoxin (P0DQQ2) from *N. melanoleuca* is also known as Nm8.

In preferred embodiments, the antigen-binding protein of the invention are capable of binding at least two α-neurotoxins selected from the group consisting of: a long neurotoxin 2 (P01388) from *N. melanoleuca* and a long neurotoxin homologous to OH-55 from *O. hannah*.

In some embodiments, the antigen-binding protein of the invention are capable of binding at least two α-neurotoxins from one or more elapid snake(s).

In other embodiments, the elapid snake(s) is (are) from the genera *Aspidelaps, Boulengerina, Bungarus, Calliophis, Dendroaspis, Elapsoidea, Hemachatus, Hemibungarus, Micruroides, Micrurus, Naja, Ophiophagus, Paranaja, Pseudohaje* and/or *Walterinnesia*.

In preferred embodiments of the present invention, the elapid snake(s) is (are) from the genera *Bungarus, Dendroaspis, Naja*, and/or *Ophiophagus*.

In other embodiments, the snake(s) is (are) Naja kaouthia, Naja melanoleuca, Naja Naja, Dendroaspis polylepis, Ophiophagus Hannah and/or Bungarus multicinctus.

The skilled person will appreciate that α-neurotoxins are antagonists of nicotinic acetylcholine receptors (nAChRs).

In some embodiments of the present invention, the antigen-binding protein is capable of blocking or neutralizing the interaction between the nicotinic acetylcholine receptor and two or more different α-neurotoxins.

In other embodiments, the antigen-binding protein is capable of binding to, blocking, or neutralizing whole snake venom.

Antibody structures, sequences, and modifications

The invention relates to an antigen-binding protein binding α-neurotoxins. The antigen-binding proteins may in particular comprise or consist of a heavy chain variable (VH) region comprising a heavy chain complementarity-determining region 3 (CDR-H3) comprising or consisting of any of SEQ ID NO: 19, 11, 27, 35, 43, and 51 or a variant thereof, wherein in said variant up to 3 amino acids have been altered for another amino acid, for example wherein 2 or 1 amino acid(s) have been altered for another amino acid.

In one embodiment, the antigen-binding protein as described herein functionally, for example in the previous sections *Antigen-binding protein capable of binding to, blocking or neutralizing two or more different α-neurotoxins* and *α-neurotoxins from snake venom* comprises the structural sequences described in this section.

In another embodiment, the antigen-binding protein comprises or consists of:

a) a heavy chain variable (VH) region comprising a heavy chain complementarity-determining region 3 (CDR-H3) comprising or consisting of any of SEQ ID NO: 19, 11, 27, 35, 43, and 51 or a variant thereof, wherein in

said variant up to 3 amino acids have been altered for another amino acid, for example wherein 2 or 1 amino acid(s) have been altered for another amino acid; and

b) a light chain variable (VL) region comprising a light chain complementarity-determining region 3 (CDR-L3) comprising or consisting of any of SEQ ID NO: 23, 15, 31, 39, 47, and 55 or a variant thereof, wherein in said variant up to 3 amino acids have been altered for another amino acid, for example wherein 2 or 1 amino acid(s) have been altered for another amino acid.

In another embodiment, the antigen-binding protein comprises or consists of:

a) a heavy chain variable (VH) region comprising:

i. a heavy chain complementarity-determining region 1 (CDR-H1) comprising or consisting of any of SEQ ID NO: 17, 9, 25, 33, 41, and 49 or a variant thereof, wherein in said variant up to 3 amino acids have been altered for another amino acid, for example wherein 2 or 1 amino acid(s) have been altered for another amino acid;

ii. a heavy chain complementarity-determining region 2 (CDR-H2) comprising or consisting of any of SEQ ID NO: 18, 10, 26, 34, 42, and 50 or a variant thereof, wherein in said variant up to 3 amino acids have been altered for another amino acid, for example wherein 2 or 1 amino acid(s) have been altered for another amino acid; and

iii. a heavy chain complementarity-determining region 3 (CDR-H3) comprising or consisting of any of SEQ ID NO: 19, 11, 27, 35, 43, and 51 or a variant thereof, wherein in said variant up to 3 amino acids have been alteredfor another amino acid, for example wherein 2 or 1 amino acid(s) have been altered for another amino acid; and

b) a light chain variable (VL) region comprising:

i. a light chain complementarity-determining region 1 (CDR-L1) comprising or consisting of any of SEQ ID NO: 21, 13, 29, 37, 45, and 53 or a variant thereof, wherein in said variant up to 3 amino acids have been altered for another amino acid, for example wherein 2 or 1 amino acid(s) have been altered for another amino acid;

ii. a light chain complementarity-determining region 2 (CDR-L2) comprising or consisting of any of SEQ ID NO: 22, 14, 30, 38, 46, and 54 or a variant thereof, wherein in said variant up to 3 amino acids

have been altered for another amino acid, for example wherein 2 or 1 amino acid(s) have been altered for another amino acid; and

iii. a light chain complementarity-determining region 3 (CDR-L3) comprising or consisting of any of SEQ ID NO: 23, 15, 31, 39, 47, and 55 or a variant thereof, wherein in said variant up to 3 amino acids have been altered for another amino acid, for example wherein 2 or 1 amino acid(s) have been altered for another amino acid.

The antigen-binding protein may be the antigen-binding protein defined as *2554_01_D11* herein.

In an embodiment, the antigen-binding protein comprises or consists of:

a) a heavy chain variable (VH) region comprising:

ii. a heavy chain complementarity-determining region 1 (CDR-H1) comprising or consisting of SEQ ID NO: 17 or a variant thereof, wherein in said variant up to 3 amino acids have been altered for another amino acid, for example wherein 2 or 1 amino acid(s) have been altered for another amino acid;

iii. a heavy chain complementarity-determining region 2 (CDR-H2) comprising or consisting of SEQ ID NO: 18 or a variant thereof, wherein in said variant up to 3 amino acids have been altered for another amino acid, for example wherein 2 or 1 amino acid(s) have been altered for another amino acid; and

iv. a heavy chain complementarity-determining region 3 (CDR-H3) comprising or consisting of SEQ ID NO: 19 or a variant thereof, wherein in said variant up to 3 amino acids have been altered for another amino acid, for example wherein 2 or 1 amino acid(s) have been altered for another amino acid; and

b) a light chain variable (VL) region comprising:

i. a light chain complementarity-determining region 1 (CDR-L1) comprising or consisting of SEQ ID NO: 21 or a variant thereof, wherein in said variant up to 3 amino acids have been altered for another amino acid, for example wherein 2 or 1 amino acid(s) have been altered for another amino acid;

ii. a light chain complementarity-determining region 2 (CDR-L2) comprising or consisting of SEQ ID NO: 22 or a variant thereof, wherein in said variant up to 3 amino acids have been altered for another amino acid, for example wherein 2 or 1 amino acid(s) have been altered for another amino acid; and

iii. a light chain complementarity-determining region 3 (CDR-L3) comprising or consisting of SEQ ID NO: 23 or a variant thereof, wherein in said variant up to 3 amino acids have been altered for another amino acid, for example wherein 2 or 1 amino acid(s) have been altered for another amino acid.

The antigen-binding protein may be the antigen-binding protein defined as *2551_01_A12* herein.

In one embodiment, the antigen-binding protein comprises or consists of:

a) a heavy chain variable (VH) region comprising:

i. a heavy chain complementarity-determining region 1 (CDR-H1) comprising or consisting of SEQ ID NO: 9 or a variant thereof, wherein in said variant up to 3 amino acids have been altered for another amino acid, for example wherein 2 or 1 amino acid(s) have been altered for another amino acid;

ii. a heavy chain complementarity-determining region 2 (CDR-H2) comprising or consisting of SEQ ID NO: 10 or a variant thereof, wherein in said variant up to 3 amino acids have been altered for another amino acid, for example wherein 2 or 1 amino acids have been altered for another amino acid; and

iii. a heavy chain complementarity-determining region 3 (CDR-H3) comprising or consisting of SEQ ID NO: 11 or a variant thereof, wherein in said variant up to 3 amino acids have been altered for another amino acid, for example wherein 2 or 1 amino acids have been altered for another amino acid; and

b) a light chain variable (VL) region comprising:

i. a light chain complementarity-determining region 1 (CDR-L1) comprising or consisting of SEQ ID NO: 13 or a variant thereof, wherein in said variant up to 3 amino acids have been altered for

another amino acid, for example wherein 2 or 1 amino acid(s) have been altered for another amino acid;

ii. a light chain complementarity-determining region 2 (CDR-L2) comprising or consisting of SEQ ID NO: 14 or a variant thereof, wherein in said variant up to 3 amino acids have been altered for another amino acid, for example wherein 2 or 1 amino acid(s) have been altered for another amino acid; and

iii. a light chain complementarity-determining region 3 (CDR-L3) comprising or consisting of SEQ ID NO: 15 or a variant thereof, wherein in said variant up to 3 amino acids have been altered for another amino acid, for example wherein 2 or 1 amino acid(s) have been altered for another amino acid.

The antigen-binding protein may be the antigen-binding protein defined as *2551_01_B11* herein.

In another embodiment, the antigen-binding protein comprises or consists of:

a) a heavy chain variable (VH) region comprising:

i. a heavy chain complementarity-determining region 1 (CDR-H1) comprising or consisting of SEQ ID NO: 25 or a variant thereof, wherein in said variant up to 3 amino acids have been altered for another amino acid, for example wherein 2 or 1 amino acid(s) have been altered for another amino acid;

ii. a heavy chain complementarity-determining region 2 (CDR-H2) comprising or consisting of SEQ ID NO: 26 or a variant thereof, wherein in said variant up to 3 amino acids have been altered for another amino acid, for example wherein 2 or 1 amino acid(s) have been altered for another amino acid; and

iii. a heavy chain complementarity-determining region 3 (CDR-H3) comprising or consisting of SEQ ID NO: 27 or a variant thereof, wherein in said variant up to 3 amino acids have been altered for another amino acid, for example wherein 2 or 1 amino acids have been altered for another amino acid; and

b) a light chain variable (VL) region comprising:

i. a light chain complementarity-determining region 1 (CDR-L1) comprising or consisting of SEQ ID NO: 29 or a variant thereof, wherein in said variant up to 3 amino acids have been altered for another amino acid, for example wherein 2 or 1 amino acid(s) have been altered for another amino acid;

ii. a light chain complementarity-determining region 2 (CDR-L2) comprising or consisting of SEQ ID NO: 30 or a variant thereof, wherein in said variant up to 3 amino acids have been altered for another amino acid, for example wherein 2 or 1 amino acid(s) have been altered for another amino acid; and

iii. a light chain complementarity-determining region 3 (CDR-L3) comprising or consisting of SEQ ID NO: 31 or a variant thereof, wherein in said variant up to 3 amino acids have been altered for another amino acid, for example wherein 2 or 1 amino acid(s) have been altered for another amino acid.

The antigen-binding protein may be the antigen-binding protein defined as *2555_01_A01* herein.

In another embodiment, the antigen-binding protein comprises or consists of:

a) a heavy chain variable (VH) region comprising:

i. a heavy chain complementarity-determining region 1 (CDR-H1) comprising or consisting of SEQ ID NO: 33 or a variant thereof, wherein in said variant up to 3 amino acids have been altered for another amino acid, for example wherein 2 or 1 amino acid(s) have been altered for another amino acid;

ii. a heavy chain complementarity-determining region 2 (CDR-H2) comprising or consisting of SEQ ID NO: 34 or a variant thereof, wherein in said variant up to 3 amino acids have been altered for another amino acid, for example wherein 2 or 1 amino acid(s) have been altered for another amino acid; and

iii. a heavy chain complementarity-determining region 3 (CDR-H3) comprising or consisting of SEQ ID NO: 35 or a variant thereof, wherein in said variant up to 3 amino acids have been altered for

another amino acid, for example wherein 2 or 1 amino acid(s) have been altered for another amino acid; and

b) a light chain variable (VL) region comprising:

i. a light chain complementarity-determining region 1 (CDR-L1) comprising or consisting of SEQ ID NO: 37 or a variant thereof, wherein in said variant up to 3 amino acids have been altered for another amino acid, for example wherein 2 or 1 amino acid(s) have been altered for another amino acid;

ii. a light chain complementarity-determining region 2 (CDR-L2) comprising or consisting of SEQ ID NO: 38 or a variant thereof, wherein in said variant up to 3 amino acids have been altered for another amino acid, for example wherein 2 or 1 amino acid(s) have been altered for another amino acid; and

iii. a light chain complementarity-determining region 3 (CDR-L3) comprising or consisting of SEQ ID NO: 39 or a variant thereof, wherein in said variant up to 3 amino acids have been altered for another amino acid, for example wherein 2 or 1 amino acid(s) have been altered for another amino acid.

The antigen-binding protein may be the antigen-binding protein defined as *2555_01_A04* herein.

In an embodiment, the antigen-binding protein comprises or consists of:

a) a heavy chain variable (VH) region comprising:

i. a heavy chain complementarity-determining region 1 (CDR-H1) comprising or consisting of SEQ ID NO: 41 or a variant thereof, wherein in said variant up to 3 amino acids have been altered for another amino acid, for example wherein 2 or 1 amino acid(s) have been altered for another amino acid;

ii. a heavy chain complementarity-determining region 2 (CDR-H2) comprising or consisting of SEQ ID NO: 42 or a variant thereof, wherein in said variant up to 3 amino acids have been altered for another amino acid, for example wherein 2 or 1 amino acid(s) have been altered for another amino acid; and

iii. a heavy chain complementarity-determining region 3 (CDR-H3) comprising or consisting of SEQ ID NO: 43 or a variant thereof, wherein in said variant up to 3 amino acids have been altered for another amino acid, for example wherein 2 or 1 amino acid(s) have been altered for another amino acid; and

b) a light chain variable (VL) region comprising:

i. a light chain complementarity-determining region 1 (CDR-L1) comprising or consisting of SEQ ID NO: 45 or a variant thereof, wherein in said variant up to 3 amino acids have been altered for another amino acid, for example wherein 2 or 1 amino acid(s) have been altered for another amino acid;

ii. a light chain complementarity-determining region 2 (CDR-L2) comprising or consisting of SEQ ID NO: 46 or a variant thereof, wherein in said variant up to 3 amino acids have been altered for another amino acid, for example wherein 2 or 1 amino acid(s) have been altered for another amino acid; and

iii. a light chain complementarity-determining region 3 (CDR-L3) comprising or consisting of SEQ ID NO: 47 or a variant thereof, wherein in said variant up to 3 amino acids have been altered for another amino acid, for example wherein 2 or 1 amino acid(s) have been altered for another amino acid.

The antigen-binding protein may be the antigen-binding protein defined as *2558_02_G09* herein.

In another embodiment, the antigen-binding protein comprises or consists of:

a) a heavy chain variable (VH) region comprising:

i. a heavy chain complementarity-determining region 1 (CDR-H1) comprising or consisting of SEQ ID NO: 49 or a variant thereof, wherein in said variant up to 3 amino acids have been altered for another amino acid, for example wherein 2 or 1 amino acid(s) have been altered for another amino acid;

ii. a heavy chain complementarity-determining region 2 (CDR-H2) comprising or consisting of SEQ ID NO: 50 or a variant thereof, wherein in said variant up to 3 amino acids have been altered for

another amino acid, for example wherein 2 or 1 amino acid(s) have been altered for another amino acid; and

iii. a heavy chain complementarity-determining region 3 (CDR-H3) comprising or consisting of SEQ ID NO: 51 or a variant thereof, wherein in said variant up to 3 amino acids have been altered for another amino acid, for example wherein 2 or 1 amino acid(s) have been altered for another amino acid; and

b) a light chain variable (VL) region comprising:

i. a light chain complementarity-determining region 1 (CDR-L1) comprising or consisting of SEQ ID NO: 53 or a variant thereof, wherein in said variant up to 3 amino acids have been altered for another amino acid, for example wherein 2 or 1 amino acid(s) have been altered for another amino acid;

ii. a light chain complementarity-determining region 2 (CDR-L2) comprising or consisting of SEQ ID NO: 54 or a variant thereof, wherein in said variant up to 3 amino acids have been altered for another amino acid, for example wherein 2 or 1 amino acid(s) have been altered for another amino acid; and

iii. a light chain complementarity-determining region 3 (CDR-L3) comprising or consisting of SEQ ID NO: 55 or a variant thereof, wherein in said variant up to 3 amino acids have been altered for another amino acid, for example wherein 2 or 1 amino acid(s) have been altered for another amino acid.

In one embodiment, the antigen-binding protein comprises or consists of:

a) a heavy chain variable (VH) region comprising or consisting of any of SEQ ID NO: 16, 8, 24, 32, 40, and 48 or a variant thereof, wherein in said variant up to 5 amino acids have been altered for another amino acid, for example wherein 4, 3, 2, or 1 amino acid(s) have been altered for another amino acid; and

b) a light chain variable (VL) region comprising or consisting of any of SEQ ID NO: 20, 12, 28, 36, 44, and 52 or a variant thereof, wherein in said variant up to 5 amino acids have been altered for another amino acid, for example wherein 4, 3, 2, or 1 amino acid(s) have been altered for another amino acid.

In another embodiment, the antigen-binding protein comprises or consists of:

a) a heavy chain variable (VH) region comprising or consisting of SEQ ID NO: 16, or a variant thereof, wherein in said variant up to 5 amino acids have been altered for another amino acid, for example wherein 4, 3, 2, or 1 amino acid(s) have been altered for another amino acid; and

b) a light chain variable (VL) region comprising or consisting of SEQ ID NO: 20, or a variant thereof, wherein in said variant up to 5 amino acids have been altered for another amino acid, for example wherein 4, 3, 2, or 1 amino acid(s) have been altered for another amino acid.

In one embodiment, the antigen-binding protein comprises or consists of:

a) a heavy chain variable (VH) region comprising or consisting of SEQ ID NO: 8, or a variant thereof, wherein in said variant up to 5 amino acids have been altered for another amino acid, for example wherein 4, 3, 2, or 1 amino acid(s) have been altered for another amino acid; and

b) a light chain variable (VL) region comprising or consisting of SEQ ID NO: 12, or a variant thereof, wherein in said variant up to 5 amino acids have been altered for another amino acid, for example wherein 4, 3, 2, or 1 amino acid(s) have been altered for another amino acid.

In one embodiment, the antigen-binding protein comprises or consists of:

a) a heavy chain variable (VH) region comprising or consisting of SEQ ID NO: 24, or a variant thereof, wherein in said variant up to 5 amino acids have been altered for another amino acid, for example wherein 4, 3, 2, or 1 amino acid(s) have been altered for another amino acid; and

b) a light chain variable (VL) region comprising or consisting of SEQ ID NO: 28, or a variant thereof, wherein in said variant up to 5 amino acids have been altered for another amino acid, for example wherein 4, 3, 2, or 1 amino acid(s) have been altered for another amino acid.

In another embodiment, the antigen-binding protein comprises or consists of:

a) a heavy chain variable (VH) region comprising or consisting of SEQ ID NO: 32, or a variant thereof, wherein in said variant up to 5 amino acids

have been altered for another amino acid, for example wherein 4, 3, 2, or 1 amino acid(s) have been altered for another amino acid; and

b) a light chain variable (VL) region comprising or consisting of SEQ ID NO: 36, or a variant thereof, wherein in said variant up to 5 amino acids have been altered for another amino acid, for example wherein 4, 3, 2, or 1 amino acid(s) have been altered for another amino acid.

In one embodiment the antigen-binding protein comprises or consists of:

a) a heavy chain variable (VH) region comprising or consisting of SEQ ID NO: 40, or a variant thereof, wherein in said variant up to 5 amino acids have been altered for another amino acid, for example wherein 4, 3, 2, or 1 amino acid(s) have been altered for another amino acid; and

b) a light chain variable (VL) region comprising or consisting of SEQ ID NO: 44, or a variant thereof, wherein in said variant up to 5 amino acids have been altered for another amino acid, for example wherein 4, 3, 2, or 1 amino acid(s) have been altered for another amino acid.

In another embodiment, the antigen-binding protein comprises or consists of:

a) a heavy chain variable (VH) region comprising or consisting of SEQ ID NO: 48, or a variant thereof, wherein in said variant up to 5 amino acids have been altered for another amino acid, for example wherein 4, 3, 2, or 1 amino acid(s) have been altered for another amino acid; and

b) a light chain variable (VL) region comprising or consisting of SEQ ID NO: 52, or a variant thereof, wherein in said variant up to 5 amino acids have been altered for another amino acid, for example wherein 4, 3, 2, or 1 amino acid(s) have been altered for another amino acid.

In some embodiments, the one or more amino acids of the antigen-binding protein that have been altered for another amino acid is (are) an amino acid(s) not part of the CDR as defined herein. In preferred embodiments, however, the antigen-binding proteins of the invention comprise the specific CDR sequences provided herein.

As used herein "signal peptide" refers to a short (less than 60 amino acids, for example, 3 to 60 amino acids) polypeptide present on precursor proteins (typically at the N terminus), and which is typically absent from the mature protein. The signal

peptide is typically rich in hydrophobic amino acids. The signal peptide directs the transport and/or secretion of the translated protein through the membrane. Signal peptides may also be called targeting signals, transit peptides, localization signals, or signal sequences. For example, the signal sequence may be a co-translational or post-translational signal peptide.

The skilled person will appreciate that it may be beneficial to improve the secretion of antigen-binding protein during its production in host cell lines, for instance during its production in the Chinese hamster ovary (CHO) cell line. Improving the secretion efficiency of the recombinant antigen-binding protein may improve its final yield.

In some embodiments, the VL and/or the VH region of the antigen-binding protein further comprises an N-terminal signal peptide sequence.

In one embodiment, the antigen-binding protein comprises an immunoglobulin constant region.

In preferred embodiments, the antigen-binding protein is selected from the group consisting of a full-length antibody, a Fab fragment, a F(ab') fragment, a $F(ab')_2$ fragment, an scFv, a diabody, and a triabody.

The antigen-binding protein may be a monoclonal antibody, preferably a human antibody or a chimeric antibody, preferably a human antibody.The antibody may be from recombinant sources and/or produced in transgenic animals. The term " antigen-binding protein" as used herein is also intended to include Fab, Fab', $F(ab')_2$, scFv, dsFv, ds-scFv, dimers, minibodies, diabodies, and multimers thereof and bispecific antibody fragments. Antibodies can be fragmented using conventional techniques. For example, $F(ab')_2$ fragments can be generated by treating the antibody with pepsin. The resulting $F(ab')_2$ fragment can be treated to reduce disulfide bridges to produce Fab' fragments. Papain digestion can lead to the formation of Fab fragments. Fab, Fab' and $F(ab')_2$, scFv, dsFv, ds-scFv, dimers, minibodies, diabodies, bispecific antibody fragments, and other fragments can also be synthesized by recombinant techniques. The antibodies are optionally in any useful isotype, including IgM, IgA, such as IgA1, IgA2, IgD, IgE or IgG, such as IgG1, IgG2, IgG3 or IgG4.

Thus, in some embodiments, the antigen-binding protein comprises an immunoglobulin constant region selected from IgG, IgM, IgA, IgD, or IgE.

In some other embodiments, the antigen-binding protein comprises an immunoglobulin constant region selected from IgG and IgA.

In other embodiments, the antigen-binding protein comprises an immunoglobulin constant region selected from IgG1, IgA1, and IgA2.

In another embodiment, the antigen-binding protein comprises an immunoglobulin constant region selected from IgG1, IgG2, IgG3, and IgG4.

In preferred embodiments, the antigen-binding protein is a monoclonal antibody.

In yet other embodiments, the antigen-binding protein is a human antibody or a chimeric antibody.

The skilled person will appreciate that antigen-binding proteins may be modified for improving their stability *in vitro* and/or *in vivo*. For example the antigen binding proteins may be modified to improve circulating half-life, such as for example when used *in vivo*. Increased antigen-binding protein half-life may improve for instance its distribution, efficacy and duration of its functional effect. Such modifications include conjugation with molecules providing a higher molecular weight and/or hydrodynamic radius to the antigen-binding protein, such as PEGylation, polysialylation or conjugation to any other protein or small molecule known to be suitable by the skilled person. The modification may also be associated with increased evasion from the immune system, thereby also increasing the circulatory time *in vivo*. Other approaches which may improve the antigen-binding protein half-life include engineering of the Fc region, such as for example a mutation of the Fc region associated with reduced antigen-binding turnover *in vivo*. The antigen-binding protein, in particular recombinant antigen-binding proteins, may also for instance be modified to improve their biological function and efficacy. The skilled person will appreciate that several post-translational modifications, such as glycosylations may have a critical impact on the efficacy of antigen-binding proteins, for example impacting their antigen binding capabilities or effector functions. For instance

the glycosylations may be glycosylations of the Fc region or the Fab of the antigen-binding protein, such as one or more N-glycosylations.

Thus in some embodiments, the antigen-binding protein has one or more modification(s) selected from the group consisting of PEGylation, polysialylation, Fc region mutation, and N-glycosylation.

The skilled person will appreciate that it may be beneficial to be able to detect and follow the antigen-binding protein for some applications. For instance conjugating the antigen-binding protein with a detection label, or detection agent, may be useful for diagnostic purposes, imaging purposes, multiplexing assays and other assays known to the skilled person. The label may be a primary label, a secondary label or any combinations thereof known to be suitable by the skilled person.

Thus, in one embodiment, the antigen-binding protein comprises a detection label.

In some applications, for instance spectroscopic assays, colorimetric assays, and light microscopy, it may be beneficial to use a colored or a luminescent dye, for instance chromophores and phosphors or any dye known to be suitable by the skilled person. Other applications such as for example fluorescent microscopic applications, flow cytometry and fluorescence-activated cell sorting (FACS), hybridization assays, or any immunoassays known to the skilled person may require or benefit from an antigen-binding protein comprising a fluorescent label or moiety. The detection label may also be a magnetic or paramagnetic label, for instance for magnetic separation application or immunoassays known to the skilled person.

In some embodiments, the antigen-binding protein label is selected from the group consisting of a colorimetric, a fluorescent, a luminescent, a magnetic, and a paramagnetic label.

The skilled person will also appreciate that other labels which can be used for instance for separation and purification, as well as for example detection in immunoassays, include the biotin-streptavidin pair. The antigen-binding protein may be biotinylated for instance for avidin (or streptavidin) detection.

In some embodiments, the detection label on the antigen-binding protein is biotin.

For other applications, such as electron microscopy, flow cytometry and lateral flow immunoassays, the skilled person will appreciate that conjugation with gold nanoparticles can be used for the detection and/or visualisation of the presence or binding or the antigen-binding protein.

In some embodiments of the present invention, the detection label is a gold nanoparticle

Preparation of antigen-binding protein

The antigen-binding proteins according to the invention may be prepared by any useful method. In one embodiment, the antibodies are prepared by recombinant methods comprising cultivating a host cell comprising a nucleic acid encoding the antigen-binding protein under control of an element directing expression in said host cell.

The antibodies are preferably identified using phase display, although other methods may also be employed. Useful methods include the methods described in **Refs. 1, 2, 3, 4, 7, and 22**. More preferably, the antigen-binding protein is identified using the methods described in Example 1 below.

Pharmaceutical compositions and combinations of antigen-binding proteins with other agents

Another aspect of the present invention relates to a pharmaceutical composition comprising an antigen-binding protein as defined herein, and a pharmaceutically acceptable diluent, carrier and/or excipient.

The antigen-binding proteins of the present invention may be combined in formulations comprising other antigen-binding protein(s) capable of binding to, blocking or neutralizing one or more snake venom antigen(s). The other antigen-binding protein(s) capable of binding to, blocking or neutralizing one or more snake venom antigen(s) may be for instance existing commercial antigen-binding protein-based antivenoms, such as the antivenoms listed in **Ref 21**, Supplementary Table 1.

In other embodiments, the pharmaceutical composition further comprises one or more additional antigen-binding protein(s) capable of binding to, blocking or neutralizing one or more snake venom antigen(s).

A further aspect of the present invention relates to a kit-of-parts comprising:

a) an antigen-binding protein as described herein; and
b) another agent suitable for the treatment of snake envenomation.

Antigen-binding proteins

In some embodiments, the other agent used for the treatment of snake envenomation in the kit-of-parts is one or more additional antigen-binding protein(s) selected from the group consisting of : IgG, IgM, scFv, $F(ab)_2$, Fab, Fab', V_HH antigen-binding protein(s) and fragments thereof.

The skilled person will know, as also mentioned in the background section of this document, that antivenom preparations, containing antigen-binding protein(s) capable of neutralizing one or more snake venom antigen(s) are traditionally obtained from hyperimmune equine, ovine, rabbit or camelid organisms. Several equine, ovine, rabbit or camelid-plasma derived antigen-binding proteins, or fragments thereof, may thus be combined with the antigen-binding protein as described herein.

In further embodiments, the one or more additional antigen-binding protein(s) capable of neutralizing one or more snake venom antigen(s) is (are) selected from the group consisting of: Equine immunoglobulin(s) against snake venom, such as Equine IgG(s) or Equine Fab or Equine $F(ab')_2$ fragment(s) against snake venom, Ovine immunoglobulin(s) against snake venom, such as Ovine IgG(s) or Ovine Fab or Ovine $F(ab')_2$ fragment(s) against snake venom, Rabbit immunoglobulin(s) against snake venom, such as Rabbit IgG(s) or Rabbit Fab or Rabbit $F(ab')_2$ fragment(s) against snake venom, and Camelids immunoglobulin(s) against snake venom, such as Camelids IgG(s) or Camelids Fab or Camelids $F(ab')_2$fragment(s) against snake venom.

Neutralizing proteins

Another type of agents which may be used for the treatment of snake envenomations are neutralizing peptides and protein inhibitors, for instance as disclosed in (**Ref. 21).**

In some other embodiments, the other agent used for the treatment of snake envenomation is one or more protein(s) displaying neutralization potential against snake venoms.

In preferred embodiments, the one or more protein(s) displaying neutralization potential against snake venoms is (are) selected from the group consisting of : a PLA_2 inhibitor, a matrix metalloproteinase (MMP) inhibitor, a snake venom metalloproteinase inhibitor (SVMP), a Kunitz-type trypsin inhibitor, an antimyotoxic factor, an antimyonecrotic factor, an antineurotoxic factor, an antihæmmoragic factor, an antioedematogenic factor and a glycoprotein, such as acid-α1-glycoprotein.

Small molecules

A further type of agents used in the treatment of snake envenomation are small molecules, for instance as described in (**Ref 21).**

In some embodiments, the other agent used for the treatment of snake envenomation is one or more small molecule(s) with inhibitory effect against snake toxins.

In further embodiments, the one or more small molecule(s) with inhibitory effect against snake toxins is (are) selected from the group consisting of: polyanions, polyphenolic compounds, and metalloproteinase inhibitors.

In preferred embodiments, the one or more small molecule(s) with inhibitory effect against snake toxins is (are) selected from the list consisting of : Suramin, Heparin, Wedelolcatone, Coumestrol, Glycyrrhizin, Fucoidan, Apigenin analogue, AG-3340, Aristolochic acid, Rosmarinic acid, Imidazopyridine, BAPTA, TPEN, EDTA, DMPS, dimercaprol, BAY-129566, Marimastat, CGS-27023A, Batimastat, Nafamostat, MV 8612 from Mandevilla velutina, MV 8608 from Mandevilla velutina, Koninginin, 4-nerolidylcatechol, Anisodamine, 12-methoxy-4-methylvoachalotine (MMV), Quinonoid

xanthene ehretianone, Benzoylsalireposide, Salireposide, Chlorogenic acid, Caffeic acid, 2-hydroxy-4-methoxy benzoic acid, and Varespladib.

Treatment of snake envenomation

Another aspect of the present invention relates to an antigen-binding protein as described herein, a pharmaceutical composition as described herein or a kit-of-parts as described herein for use in a method of treatment of snake envenomation in a subject.

In a preferred embodiment, the antigen-binding protein, the pharmaceutical composition or the kit-of-parts for the use as described herein prevents or delay venom-induced lethality.

In one embodiment, the antigen-binding protein as described herein prevents venom-induced lethality in a human being or in a mouse after said human being or said mouse has received an otherwise lethal dosis, such as two LD50s, of any of the snake venoms e.g. of *D. polylepis*. *N. kaouthia* and/or *O. hannah* comprising one or more α-neurotoxins described herein, e.g. α- cobratoxin.

In one embodiment, the antigen-binding protein as described herein delays venom-induced lethality in a human being or in a mouse for at least 8h, such as at least 10h, for instance at least 12h, such as at least 1 day, for instance at least 2 days, such as at least 3 days after said human being or said mouse has received an otherwise lethal dosis of a snake venom and/or α-neurotoxins as described herein, e.g. of α-cobratoxin.

In other embodiments, the antigen-binding protein, the pharmaceutical composition or the kit-of-parts for the use as described herein, prevents or delays envenomation symptoms.

In further embodiments, the antigen-binding protein, the pharmaceutical composition or the kit-of-parts prevents or delays envenomation symptoms.
The skilled person will appreciate that the main effect of snake envenomation, in particular snake envenomation by a venom containing α-neurotoxins, is paralysis. Several other direct and indirect symptoms can occur as a result to snake envenomation.

In some embodiments, the envenomation symptoms comprise limb paralysis, respiratory paralysis, redness, swelling, bruising, bleeding, blistering, severe pain at the site of the bite, tenderness at the site of the bite, nausea, vomiting, diarrhea, rapid heart rate, weak pulse, low blood pressure, disturbed vision, increased salivation, increased sweating, numbness, tingling and/or muscle twitching. Preferably, the antigen-binding proteins are useful for treatment of paralysis, such as limb paralysis, and/or respiratory paralysis caused by snake envenomation.

In preferred embodiments, the antigen-binding protein, the pharmaceutical composition or the kit-of-parts as described herein is administered parenterally or pulmonarily.

In yet other embodiments, the parenteral administration of the antigen-binding protein, the pharmaceutical composition or the kit-of-parts is intravenous, intramuscular and/or sub-cutaneous.

The skilled person will appreciate that antivenoms should ideally be provided to the subject in need thereof as soon as possible after the bite. Antivenoms may however not be available close to the location of the envenomation event, in particular when the snake bite occurs in remote geographical areas, for example in geographical areas with limited vehicle access, complicated logistics, and/or scarce healthcare center presence or healthcare resources. It may thus be of great benefit that the antigen-binding protein, the pharmaceutical composition or the kit-of-parts for use in the treatment of snake envenomation can be administered shortly after the bite but also may have a preventing or delaying action on the symptoms or a fortiori on lethality when administered relatively longer after the bite.

In some embodiments, the antigen-binding protein, the pharmaceutical composition or the kit-of-parts is administered no more than 5 min after envenomation, such as no more than 10 min after envenomation, for instance no more than 15 min after envenomation, such as no more than 20 min after envenomation, for instance no more than 30 min after envenomation, such as no more than 45 min after envenomation, for instance no more than 60 min after envenomation, such as no more than 90 min after envenomation, for instance no more than 120 min after envenomation, such as no more than 180 min after envenomation, for instance no more than 240 min after

envenomation, such as no more than 6h after envenomation, for instance no more than 12h after envenomation, such as no more than 24h after envenomation, for instance no more than 48h after envenomation, such as no more than 72h after envenomation, for instance no more than 96h after envenomation, such as no more than 120h after envenomation for instance no more than 144h after envenomation.

In another aspect, the present invention relates to a method of treating snake envenomation which comprises administering to a subject an effective amount of the antigen-binding protein as described herein, the composition as described herein, or the kit-of-parts as described herein.

A further aspect of the present invention relates to the use of the antigen-binding protein as described herein, the composition as described herein, or the kit-of-parts as described herein, for the manufacture of a medicament for the treatment of snake envenomation.

Diagnosis of snake envenomation

The antigen-binding protein of the present invention may be used for detection, diagnosis, and diagnostics purposes, for example in clinical settings. The antigen-binding protein may be used for instance as part of a companion diagnostic kit to support for example the clinical management of snakebites and identify therapeutic options.

Another aspect of the invention relates to a kit for detection and/or quantification of snake venom in a sample comprising:

- one or more antigen-binding proteins, such as two antigen-binding proteins, wherein at least one of said antigen-binding proteins is as described herein; and
- means for detection of a complex comprising said one or more antigen-binding proteins bound to snake venom or toxins.

In some embodiments, the kit further comprises means for acquiring or isolating a sample from a subject.

In preferred embodiments, the sample is a blood sample, a urine sample or a smear of the bite site/wound.

In further embodiments, the blood sample is a plasma blood sample.

The skilled person will appreciate that the kit may be an immunoassay, such as a method for detecting one or more analytes, such as snake venoms, or fractions thereof, for example α-neurotoxins. Typically, the immunoassay may be a method based on the specific binding of the antigen-binding proteins of the present invention to one or more α-neurotoxins. Suitable immunoassays include ELISA (enzyme-linked immunosorbent assay), indirect ELISA, sandwich ELISA, competitive ELISA, multiplex ELISA, radioimmunoassay (RIA), ELISPOT technologies, and other similar techniques known in the art.

In a further embodiment of the present invention, the kit is an immunoassay, such as a lateral flow assay, an ELISA, an electrochemical impedance spectroscopy-based assay, a Bilayer Interferometry (BLI)-based assay or a turbidimetric immunoassay.

Another aspect of the invention relates to an antigen-binding protein as described herein for use in the diagnosis of snake envenomation in a subject.

The antigen-binding protein may be used for instance for the diagnosis of snake envenomation with a venom containing one or more α-neurotoxins.

A further aspect of the present invention relates to the use of an antigen-binding protein as described herein in an *in vitro* method for detection and/or diagnosis of snake envenomation in a subject.

Another aspect of the present invention relates to a method for detecting snake venom in a sample, said method comprising:

a) providing a sample for analysis;
b) contacting the sample with one or more antigen-binding proteins as defined herein to said sample; and
c) detecting a complex comprising said one or more antigen-binding proteins bound to a snake antigen.

In some embodiments, the method is an *in vitro* method.

In further embodiments, the detection of the complex is performed by an immunoassay, such as a lateral flow assay, an ELISA, an electrochemical impedance spectroscopy-based assay, a Bilayer Interferometry (BLI)-based assay or a turbidimetric immunoassay.

Organisms subject to snake envenomation

The person skilled in the art will appreciate that the use of antivenoms may not be restricted to humans but can also be used on non-human animals, such as for instance pets, cattle, farm animals, or ornamental animals.

In one embodiment of the present invention the subject of the antigen-binding protein, the pharmaceutical composition or the kit-of-parts for the use as described herein, the kit as described herein, or the antigen-binding protein for use as described herein is a human or a non-human animal.

In a further embodiment, the non-human animal subject belongs to the family of Equidae, Bovidae, Camelidae, Canidae, or Felidae.

In some embodiments, the non-human animal is a horse, a cow, an ox, a camel, a dog, or a cat.

Items

The invention may further be defined by anyone of the following items:

1. An antigen-binding protein capable of binding to, blocking or neutralizing two or more different α-neurotoxins.

2. An antigen-binding protein capable of binding to, blocking or neutralizing two or more different long-chain α-neurotoxins, for example at least 3, such as at least 4, for example at least 5, such as at least 6, for example at least 7 different long-chain α-neurotoxins, wherein said antigen-binding protein binds to two or more different long-chain α-neurotoxins with a Kd of at the most 10nM.

3. The antigen-binding protein according to any one of the preceding items, wherein the antigen-binding protein is capable of binding to, blocking or neutralizing at least 3, such as at least 4, for example at least 5, such as at least 6, for example at least 7 different α-neurotoxins.

4. The antigen-binding protein according to any one of the preceding items, wherein the α-neurotoxins are long-chain-α-neurotoxins.

5. The antigen-binding protein according to any one of the preceding items, wherein the antigen-binding protein is capable of binding to, blocking or neutralizing at least 3 different long-chain-α-neurotoxins.

6. The antigen-binding protein according to any one of the preceding items, wherein the α-neurotoxins are selected from the group consisting of: α-elapitoxins and α-bungarotoxins.

7. The antigen-binding protein according to any one of the preceding items, wherein at least one of said α-neurotoxins is an α-elapitoxin.

8. The antigen-binding protein according to any one of items 5 to 7, wherein at least one α-elapitoxin is α-elapitoxin-Dppd2.

9. The antigen-binding protein according to any one of the preceding items, wherein at least one α-neurotoxin is α-cobratoxin.

10. The antigen-binding protein according to anyone of the preceding items, wherein the α-neurotoxins are selected from the group consisting of: long neurotoxin 2 (A8N285) from *O. hannah*, α-cobratoxin (P01391) from *N. kaouthia*, long neurotoxin 2 (P01388) and long neurotoxin (P0DQQ2) from *N. melanoleuca*, long neurotoxin 4 (P25672) from *N. naja*, and α-elapitoxin (P01396) from *D. polylepis*.

11. The antigen-binding protein according to any one of the preceding items, wherein the α-neurotoxins are selected from the group consisting of: a long neurotoxin 2 (P01388) from *N. melanoleuca* and a long neurotoxin homologous to OH-55 from *O. hannah*.

12. The antigen-binding protein according to any one of the preceding items, wherein the two or more different long-chain α-neurotoxins comprise or consist of α-elapitoxin, α-cobratoxin, and Nm8.

13. The antigen-binding protein according to any one of the preceding items, wherein the two or more different long-chain α-neurotoxins comprise or consist of α-elapitoxin, α-cobratoxin, and long neurotoxin (P0DQQ2) from *N. melanoleuca.*

14. The antigen-binding protein according to any one of the preceding items, wherein the two or more different long-chain α-neurotoxins comprise or consist of long neurotoxin (P0DQQ2) from *N. melanoleuca*, α-cobratoxin (P01391) from *N. kaouthia* and α-elapitoxin (P01396) from *D. polylepis*.

15. The antigen-binding protein according to any one of the preceding items, wherein the α-neurotoxins are from one or more elapid snake(s).

16. The antigen-binding protein according to item 15, wherein the elapid snake(s) is (are) from the genera *Aspidelaps, Boulengerina, Bungarus, Calliophis,*

Dendroaspis, Elapsoidea, Hemachatus, Hemibungarus, Micruroides, Micrurus, Naja, Ophiophagus, Paranaja, Pseudohaje and/or *Walterinnesia*.

17. The antigen-binding protein according to item 16, wherein the elapid snake(s) is (are) from the genera *Bungarus, Dendroaspis, Naja,* and/or *Ophiophagus*.

18. The antigen-binding protein according to item 17, wherein the elapid snakes are of the genera *Naja* and/or *Dendroaspis*.

19. The antigen-binding protein according to item 17, wherein the elapid snake(s) is (are) *Naja kaouthia, Naja melanoleuca, Naja Naja, Dendroaspis polylepis, Ophiophagus Hannah* and/or *Bungarus multicinctus*.

20. The antigen-binding protein according to any one of the preceding items capable of blocking or neutralizing the interaction between the nicotinic acetylcholine receptor and two or more different α-neurotoxins.

21. The antigen-binding protein according to any one of the preceding items capable of binding to, blocking or neutralizing whole snake venom.

22. The antigen-binding protein according to any one of the preceding items, wherein said antigen-binding protein binds to two or more different α-neurotoxins with a K_d of at the most 8.5 nM, such as at the most 9 nM, for instance at the most 9.5nM, such as at the most 10 nM, for instance at the most 12.5nM, such as at the most 15 nM, for instance at the most 20nM, such as at the most 25 nM, for instance at the most 50nM, such as at the most 75 nM, for instance at the most 85nM.

23. The antigen-binding protein according to any one of the preceding items, wherein said antigen-binding protein binds to two or more different α-neurotoxins with a K_d of at the most 10 nM.

24. The antigen-binding protein according to any one of the preceding items, wherein said antigen-binding protein binds to two or more different α-neurotoxins with a K_d of at the most 5 nM.

25. An antigen-binding protein comprising or consisting of a heavy chain variable (VH) region comprising a heavy chain complementarity-determining region 3 (CDR-H3) comprising or consisting of SEQ ID NO: 19, or a variant thereof, wherein in said variant up to 3 amino acids have been altered, for example wherein 2 or 1 amino acid(s) have been altered.

26. The antigen-binding protein according to any one of items 1 to 24, wherein the antigen-binding protein comprises the sequences according to item 25.

27. The antigen-binding protein according to any one of the preceding items comprising or consisting of:

a) a heavy chain variable (VH) region comprising a heavy chain complementarity-determining region 3 (CDR-H3) comprising or consisting of any of SEQ ID NO: 19, 11, 27, 35, 43 and 51 or a variant thereof, wherein in said variant up to 3 amino acids have been altered, for example wherein 2 or 1 amino acid(s) have been altered;

and

b) a light chain variable (VL) region comprising a light chain complementarity-determining region 3 (CDR-L3) comprising or consisting of any of SEQ ID NO: 23, 15, 31, 39, 47 and 55 or a variant thereof, wherein in said variant up to 3 amino acids have been altered, for example wherein 2 or 1 amino acid(s) have been altered.

28. The antigen-binding protein according to any one of the preceding items comprising or consisting of:

c) a heavy chain variable (VH) region comprising:

i. a heavy chain complementarity-determining region 1 (CDR-H1) comprising or consisting of any of SEQ ID NO: 17, 9, 25, 33, 41 or 49 or a variant thereof, wherein in said variant up to 3 amino

acids have been altered, for example wherein 2 or 1 amino acid(s) have been altered;

ii. a heavy chain complementarity-determining region 2 (CDR-H2) comprising or consisting of any of SEQ ID NO: 18, 10, 26, 34, 42 and 50 or a variant thereof, wherein in said variant up to 3 amino acids have been altered, for example wherein 2 or 1 amino acid(s) have been altered; and

iii. a heavy chain complementarity-determining region 3 (CDR-H3) comprising or consisting of any of SEQ ID NO: 19, 11, 27, 35, 43 and 51 or a variant thereof, wherein in said variant up to 3 amino acids have been altered, for example wherein 2 or 1 amino acid(s) have been altered;

and

d) a light chain variable (VL) region comprising:

i. a light chain complementarity-determining region 1 (CDR-L1) comprising or consisting of any of SEQ ID NO: 21, 13, 29, 37, 45 and 53 or a variant thereof, wherein in said variant up to 3 amino acids have been altered, for example wherein 2 or 1 amino acid(s) have been altered;

ii. a light chain complementarity-determining region 2 (CDR-L2) comprising or consisting of any of SEQ ID NO: 22, 14, 30, 38, 46 and 54 or a variant thereof, wherein in said variant up to 3 amino acids have been altered, for example wherein 2 or 1 amino acid(s) have been altered; and

iii. a light chain complementarity-determining region 3 (CDR-L3) comprising or consisting of any of SEQ ID NO: 23, 15, 31, 39, 47 and 55 or a variant thereof, wherein in said variant up to 3 amino acids have been altered, for example wherein 2 or 1 amino acid(s) have been altered.

29. An antigen-binding protein comprising or consisting of:

a) a heavy chain variable (VH) region comprising:

i. a heavy chain complementarity-determining region 1 (CDR-H1) comprising or consisting of any of SEQ ID NO: 17, 9, 25, 33, 41 or 49 or a variant thereof, wherein in said variant up to 3 amino

acids have been altered, for example wherein 2 or 1 amino acid(s) have been altered;

ii. a heavy chain complementarity-determining region 2 (CDR-H2) comprising or consisting of any of SEQ ID NO: 18, 10, 26, 34, 42 and 50 or a variant thereof, wherein in said variant up to 3 amino acids have been altered, for example wherein 2 or 1 amino acid(s) have been altered; and

iii. a heavy chain complementarity-determining region 3 (CDR-H3) comprising or consisting of any of SEQ ID NO: 19, 11, 27, 35, 43 and 51 or a variant thereof, wherein in said variant up to 3 amino acids have been altered, for example wherein 2 or 1 amino acid(s) have been altered;

and

b) a light chain variable (VL) region comprising:

iv. a light chain complementarity-determining region 1 (CDR-L1) comprising or consisting of any of SEQ ID NO: 21, 13, 29, 37, 45 and 53 or a variant thereof, wherein in said variant up to 3 amino acids have been altered, for example wherein 2 or 1 amino acid(s) have been altered;

v. a light chain complementarity-determining region 2 (CDR-L2) comprising or consisting of any of SEQ ID NO: 22, 14, 30, 38, 46 and 54 or a variant thereof, wherein in said variant up to 3 amino acids have been altered, for example wherein 2 or 1 amino acid(s) have been altered; and

vi. a light chain complementarity-determining region 3 (CDR-L3) comprising or consisting of any of SEQ ID NO: 23, 15, 31, 39, 47 and 55 or a variant thereof, wherein in said variant up to 3 amino acids have been altered, for example wherein 2 or 1 amino acid(s) have been altered.

30. The antigen-binding protein according to any one of the preceding items, wherein the antigen-binding protein comprises or consists of:

c) a heavy chain variable (VH) region comprising:

i. a heavy chain complementarity-determining region 1 (CDR-H1) comprising or consisting of SEQ ID NO: 17 or a variant thereof,

wherein in said variant up to 3 amino acids have been altered, for example wherein 2 or 1 amino acid(s) have been altered;

ii. a heavy chain complementarity-determining region 2 (CDR-H2) comprising or consisting of SEQ ID NO: 18 or a variant thereof, wherein in said variant up to 3 amino acids have been altered, for example wherein 2 or 1 amino acid(s) have been altered; and

iii. a heavy chain complementarity-determining region 3 (CDR-H3) comprising or consisting of SEQ ID NO: 19 or a variant thereof, wherein in said variant up to 3 amino acids have been altered, for example wherein 2 or 1 amino acid(s) have been altered;

and

d) a light chain variable (VL) region comprising:

vii. a light chain complementarity-determining region 1 (CDR-L1) comprising or consisting of SEQ ID NO: 21 or a variant thereof, wherein in said variant up to 3 amino acids have been altered, for example wherein 2 or 1 amino acid(s) have been altered;

viii. a light chain complementarity-determining region 2 (CDR-L2) comprising or consisting of SEQ ID NO: 22 or a variant thereof, wherein in said variant up to 3 amino acids have been altered, for example wherein 2 or 1 amino acid(s) have been altered; and

ix. a light chain complementarity-determining region 3 (CDR-L3) comprising or consisting of SEQ ID NO: 23 or a variant thereof, wherein in said variant up to 3 amino acids have been altered, for example wherein 2 or 1 amino acid(s) have been altered.

31. The antigen-binding protein according to any one of items 1 to 29, wherein the antigen-binding protein comprises or consists of:

a) a heavy chain variable (VH) region comprising:

i. a heavy chain complementarity-determining region 1 (CDR-H1) comprising or consisting of SEQ ID NO: 9 or a variant thereof, wherein in said variant up to 3 amino acids have been altered, for example wherein 2 or 1 amino acid(s) have been altered;

ii. a heavy chain complementarity-determining region 2 (CDR-H2) comprising or consisting of SEQ ID NO: 10 or a variant thereof,

wherein in said variant up to 3 amino acids have been altered, for example wherein 2 or 1 amino acids have been altered; and

iii. a heavy chain complementarity-determining region 3 (CDR-H3) comprising or consisting of SEQ ID NO: 11 or a variant thereof, wherein in said variant up to 3 amino acids have been altered, for example wherein 2 or 1 amino acids have been altered;

and

b) a light chain variable (VL) region comprising:

i. a light chain complementarity-determining region 1 (CDR-L1) comprising or consisting of SEQ ID NO: 13 or a variant thereof, wherein in said variant up to 3 amino acids have been altered, for example wherein 2 or 1 amino acid(s) have been altered;

ii. a light chain complementarity-determining region 2 (CDR-L2) comprising or consisting of SEQ ID NO: 14 or a variant thereof, wherein in said variant up to 3 amino acids have been altered, for example wherein 2 or 1 amino acid(s) have been altered; and

iii. a light chain complementarity-determining region 3 (CDR-L3) comprising or consisting of SEQ ID NO: 15 or a variant thereof, wherein in said variant up to 3 amino acids have been altered , for example wherein 2 or 1 amino acid(s) have been altered .

32. The antigen-binding protein according to any one of items 1 to 29, wherein the antigen-binding protein comprises or consists of:

a) a heavy chain variable (VH) region comprising:

i. a heavy chain complementarity-determining region 1 (CDR-H1) comprising or consisting of SEQ ID NO: 25 or a variant thereof, wherein in said variant up to 3 amino acids have been altered , for example wherein 2 or 1 amino acid(s) have been altered ;

ii. a heavy chain complementarity-determining region 2 (CDR-H2) comprising or consisting of SEQ ID NO: 26 or a variant thereof, wherein in said variant up to 3 amino acids have been altered , for example wherein 2 or 1 amino acid(s) have been altered ; and

iii. a heavy chain complementarity-determining region 3 (CDR-H3) comprising or consisting of SEQ ID NO: 27 or a variant thereof, wherein in said variant up to 3 amino acids have been altered , for example wherein 2 or 1 amino acids have been altered ;

and

b) a light chain variable (VL) region comprising:

iv. a light chain complementarity-determining region 1 (CDR-L1) comprising or consisting of SEQ ID NO: 29 or a variant thereof, wherein in said variant up to 3 amino acids have been altered , for example wherein 2 or 1 amino acid(s) have been altered ;

v. a light chain complementarity-determining region 2 (CDR-L2) comprising or consisting of SEQ ID NO: 30 or a variant thereof, wherein in said variant up to 3 amino acids have been altered , for example wherein 2 or 1 amino acid(s) have been altered ;

and

vi. a light chain complementarity-determining region 3 (CDR-L3) comprising or consisting of SEQ ID NO: 31 or a variant thereof, wherein in said variant up to 3 amino acids have been altered , for example wherein 2 or 1 amino acid(s) have been altered .

33. The antigen-binding protein according to any one of items 1 to 29, wherein the antigen-binding protein comprises or consists of:

a) a heavy chain variable (VH) region comprising:

i. a heavy chain complementarity-determining region 1 (CDR-H1) comprising or consisting of SEQ ID NO: 33 or a variant thereof, wherein in said variant up to 3 amino acids have been altered , for example wherein 2 or 1 amino acid(s) have been altered ;

ii. a heavy chain complementarity-determining region 2 (CDR-H2) comprising or consisting of SEQ ID NO: 34 or a variant thereof, wherein in said variant up to 3 amino acids have been altered , for example wherein 2 or 1 amino acid(s) have been altered ;

and

iii. a heavy chain complementarity-determining region 3 (CDR-H3) comprising or consisting of SEQ ID NO: 35 or a variant thereof,

wherein in said variant up to 3 amino acids have been altered , for example wherein 2 or 1 amino acid(s) have been altered ;

and

b) a light chain variable (VL) region comprising:

i. a light chain complementarity-determining region 1 (CDR-L1) comprising or consisting of SEQ ID NO: 37 or a variant thereof, wherein in said variant up to 3 amino acids have been altered , for example wherein 2 or 1 amino acid(s) have been altered ;

ii. a light chain complementarity-determining region 2 (CDR-L2) comprising or consisting of SEQ ID NO: 38 or a variant thereof, wherein in said variant up to 3 amino acids have been altered , for example wherein 2 or 1 amino acid(s) have been altered ; and

iii. a light chain complementarity-determining region 3 (CDR-L3) comprising or consisting of SEQ ID NO: 39 or a variant thereof, wherein in said variant up to 3 amino acids have been altered , for example wherein 2 or 1 amino acid(s) have been altered .

34. The antigen-binding protein according to any one of items 1 to 29, wherein the antigen-binding protein comprises or consists of:

a) a heavy chain variable (VH) region comprising:

i. a heavy chain complementarity-determining region 1 (CDR-H1) comprising or consisting of SEQ ID NO: 41 or a variant thereof, wherein in said variant up to 3 amino acids have been altered , for example wherein 2 or 1 amino acid(s) have been altered ;

ii. a heavy chain complementarity-determining region 2 (CDR-H2) comprising or consisting of SEQ ID NO: 42 or a variant thereof, wherein in said variant up to 3 amino acids have been altered , for example wherein 2 or 1 amino acid(s) have been altered ; and

iii. a heavy chain complementarity-determining region 3 (CDR-H3) comprising or consisting of SEQ ID NO: 43 or a variant thereof, wherein in said variant up to 3 amino acids have been altered , for example wherein 2 or 1 amino acid(s) have been altered ;

and

b) a light chain variable (VL) region comprising:

i. a light chain complementarity-determining region 1 (CDR-L1) comprising or consisting of SEQ ID NO: 45 or a variant thereof, wherein in said variant up to 3 amino acids have been altered , for example wherein 2 or 1 amino acid(s) have been altered ;

ii. a light chain complementarity-determining region 2 (CDR-L2) comprising or consisting of SEQ ID NO: 46 or a variant thereof, wherein in said variant up to 3 amino acids have been altered , for example wherein 2 or 1 amino acid(s) have been altered ; and

iii. a light chain complementarity-determining region 3 (CDR-L3) comprising or consisting of SEQ ID NO: 47 or a variant thereof, wherein in said variant up to 3 amino acids have been altered , for example wherein 2 or 1 amino acid(s) have been altered .

35. The antigen-binding protein according to any one of items 1 to 29, wherein said antigen-binding protein comprises or consists of:

a) a heavy chain variable (VH) region comprising:

i. a heavy chain complementarity-determining region 1 (CDR-H1) comprising or consisting of SEQ ID NO: 49 or a variant thereof, wherein in said variant up to 3 amino acids have been altered , for example wherein 2 or 1 amino acid(s) have been altered ;

ii. a heavy chain complementarity-determining region 2 (CDR-H2) comprising or consisting of SEQ ID NO: 50 or a variant thereof, wherein in said variant up to 3 amino acids have been altered , for example wherein 2 or 1 amino acid(s) have been altered ; and

iii. a heavy chain complementarity-determining region 3 (CDR-H3) comprising or consisting of SEQ ID NO: 51 or a variant thereof, wherein in said variant up to 3 amino acids have been altered , for example wherein 2 or 1 amino acid(s) have been altered ;

and

b) a light chain variable (VL) region comprising:

i. a light chain complementarity-determining region 1 (CDR-L1) comprising or consisting of SEQ ID NO: 53 or a variant thereof,

wherein in said variant up to 3 amino acids have been altered , for example wherein 2 or 1 amino acid(s) have been altered ;

ii. a light chain complementarity-determining region 2 (CDR-L2) comprising or consisting of SEQ ID NO: 54 or a variant thereof, wherein in said variant up to 3 amino acids have been altered , for example wherein 2 or 1 amino acid(s) have been altered ; and

iii. a light chain complementarity-determining region 3 (CDR-L3) comprising or consisting of SEQ ID NO: 55 or a variant thereof, wherein in said variant up to 3 amino acids have been altered , for example wherein 2 or 1 amino acid(s) have been altered .

36. The antigen-binding protein according to any one of items 1 to 29, wherein the protein comprises or consists of:

a) a heavy chain variable (VH) region comprising or consisting of any of SEQ ID NO: 16, 8, 24, 32, 40 or 48 or a variant thereof, wherein in said variant up to 5 amino acids have been altered , for example wherein 4, 3, 2, or 1 amino acid(s) have been altered ; and

b) a light chain variable (VL) region comprising or consisting of any of SEQ ID NO: 20, 12, 28, 36, 44 and 52 or a variant thereof, wherein in said variant up to 5 amino acids have been altered , for example wherein 4, 3, 2, or 1 amino acid(s) have been altered .

37. The antigen-binding protein according to any one of items 1 to 29, wherein the protein comprises or consists of:

a) a heavy chain variable (VH) region comprising or consisting of SEQ ID NO: 16, or a variant thereof, wherein in said variant up to 5 amino acids have been altered , for example wherein 4, 3, 2, or 1 amino acid(s) have been altered ; and

c) a light chain variable (VL) region comprising or consisting of SEQ ID NO: 20, or a variant thereof, wherein in said variant up to 5 amino acids have been altered, for example wherein 4, 3, 2, or 1 amino acid(s) have been altered .

38. The antigen-binding protein according to any one of items 1 to 29, wherein the protein comprises or consists of:
    a) a heavy chain variable (VH) region comprising or consisting of SEQ ID NO: 8, or a variant thereof, wherein in said variant up to 5 amino acids have been altered , for example wherein 4, 3, 2, or 1 amino acid(s) have been altered ; and
    b) a light chain variable (VL) region comprising or consisting of SEQ ID NO: 12, or a variant thereof, wherein in said variant up to 5 amino acids have been altered , for example wherein 4, 3, 2, or 1 amino acid(s) have been altered .

39. The antigen-binding protein according to any one of items 1 to 29, wherein the protein comprises or consists of:
    a) a heavy chain variable (VH) region comprising or consisting of SEQ ID NO: 24, or a variant thereof, wherein in said variant up to 5 amino acids have been altered , for example wherein 4, 3, 2, or 1 amino acid(s) have been altered ; and
    b) a light chain variable (VL) region comprising or consisting of SEQ ID NO: 28, or a variant thereof, wherein in said variant up to 5 amino acids have been altered , for example wherein 4, 3, 2, or 1 amino acid(s) have been altered .

40. The antigen-binding protein according to items 1 to 29, wherein the protein comprises or consists of:
    a) a heavy chain variable (VH) region comprising or consisting of SEQ ID NO: 32, or a variant thereof, wherein in said variant up to 5 amino acids have been altered , for example wherein 4, 3, 2, or 1 amino acid(s) have been altered ; and
    b) a light chain variable (VL) region comprising or consisting of SEQ ID NO: 36, or a variant thereof, wherein in said variant up to 5 amino acids have been altered , for example wherein 4, 3, 2, or 1 amino acid(s) have been altered .

41. The antigen-binding protein according to items 1 to 29, wherein the protein comprises or consists of:
    a) a heavy chain variable (VH) region comprising or consisting of SEQ ID NO: 40, or a variant thereof, wherein in said variant up to 5 amino acids have been altered , for example wherein 4, 3, 2, or 1 amino acid(s) have been altered ; and
    b) a light chain variable (VL) region comprising or consisting of SEQ ID NO: 44, or a variant thereof, wherein in said variant up to 5 amino acids have been altered , for example wherein 4, 3, 2, or 1 amino acid(s) have been altered .

42. The antigen-binding protein according to items 1 to 25, wherein the protein comprises or consists of:
    a) a heavy chain variable (VH) region comprising or consisting of SEQ ID NO: 48, or a variant thereof, wherein in said variant up to 5 amino acids have been altered , for example wherein 4, 3, 2, or 1 amino acid(s) have been altered ; and
    b) a light chain variable (VL) region comprising or consisting of SEQ ID NO: 52, or a variant thereof, wherein in said variant up to 5 amino acids have been altered , for example wherein 4, 3, 2, or 1 amino acid(s) have been altered .

43. The antigen-binding protein according to any one of items 25 to 42, wherein the one or more amino acids that have been altered is (are) an amino acid(s) not part of the CDR as defined in any one of items 25 to 35.

44. The antigen-binding protein according to any one of items 25 to 42, wherein the one or more amino acids that have been altered, have been substituted by another amino acid.

45. The antigen-binding protein according to any one of the preceding items, wherein the VL and/or the VH region further comprises an N-terminal signal peptide sequence.

46. The antigen-binding protein according to any one of the preceding items, wherein the protein is selected from the group consisting of a full-length antibody, a Fab fragment, a F(ab') fragment, a $F(ab')_2$ fragment, an scFv, a diabody, and a triabody.

47. The antigen-binding protein according to any one of the preceding items comprising an immunoglobulin constant region.

48. The antigen-binding protein according to any one of the preceding items comprising an immunoglobulin constant region selected from IgG, IgM, IgA, IgD, and IgE.

49. The antigen-binding protein according to any one of the preceding items comprising an immunoglobulin constant region selected from IgG and IgA.

50. The antigen-binding protein according to any one of the preceding items comprising an immunoglobulin constant region selected from IgG1, IgA1, and IgA2.

51. The antigen-binding protein according to any one of the preceding items comprising an immunoglobulin constant region selected from IgG1, IgG2, IgG3, and IgG4.

52. The antigen-binding protein according to any one of the preceding items, wherein the protein is a monoclonal antibody.

53. The antigen-binding protein according to any one of the preceding items, wherein the protein is a human antibody or a chimeric antibody.

54. The antigen-binding protein according to any one of the preceding items, wherein the protein has one or more modification(s) selected from the group consisting of PEGylation, polysialylation, Fc region mutation and N-glycosylation.

55. The antigen-binding protein according to any one of the preceding items, wherein the protein comprises a detection label.

56. The antigen-binding protein according to item 55, wherein the detection label is selected from the group consisting of a colorimetric, a fluorescent, a luminescent, a magnetic, and a paramagnetic label.

57. The antigen-binding protein according to any one of items 55 to 56, wherein the detection label is biotin.

58. The antigen-binding protein according to any one of items 55 to 56, wherein the detection label is a gold nanoparticle

59. The antigen-binding protein according to any one of the preceding items wherein the neutralization is obtained at a α-neurotoxin-to-antigen-binding protein molar ratio of no more than 1:1, such as no more than 1:1.5, for instance no more than 1:2, such as no more than 1:2.5, for instance no more than 1:3, such as no more than 1:3.5, for instance no more than 1:4, such as no more than 1:5.

60. The antigen-binding protein according to any one of the preceding items wherein the IC50 value of the neutralization of the α-neurotoxin by the antigen-binding protein is no more than 6 nM, such as no more than 5.5 nM, for instance no more than 5nM, such as no more than 4.5 nM, for instance no more than 4 nM, for instance no more than 3.5 nM, such as no more than 3 nM, for instance no more than 2.5 nM, such as no more than 2 nM, for instance no more than 1.5 nM, such as no more than 1 nM.

61. The antigen-binding protein according to any one of the preceding items, wherein the antigen-binding protein is capable to bind to an epitope contained between residues corresponding to amino acids 24 to 60 of the toxin protein sequences as defined in any one of SEQ:IDs No 56, 57, 58, 59 and 60 or variant(s) thereof having at least 70% identity, such as at least 75% identity, for example at least 80% identity, such as at least 85% identity, for example at least 90% identity, such as at least 95% identity thereto, preferably wherein the

antigen-binding protein is capable to bind to an epitope contained between residues corresponding to amino acids 41 to 60 of the toxin protein sequences as defined in any one of SEQ:IDs No 56, 57, 58, 59 and 60 or variant(s) thereof having at least 70% identity, such as at least 75% identity, for example at least 80% identity, such as at least 85% identity, for example at least 90% identity, such as at least 95% identity thereto.

62. The antigen-binding protein according to any one of the preceding items, wherein the antigen-binding protein is capable to bind to an epitope comprising or consisting of the sequence: CAATCPXVKXGVXIXCCST (SEQ ID NO: 69), wherein X may be any amino acid.

63. The antigen-binding protein according to any one of the preceding items, wherein the antigen-binding protein is capable of preventing or delaying venom-induced lethality in a mammal in need thereof.

64. The antigen-binding protein according to any one of the preceding items wherein the antigen-binding protein delays venom-induced lethality for at least 8h, such as at least 10h, for instance at least 12h, such as at least 1 day, for instance at least 2 days, such as at least 3 days after envenomation.

65. A pharmaceutical composition comprising an antigen-binding protein as defined in any one of the preceding items, and a pharmaceutically acceptable diluent, carrier and/or excipient.

66. The pharmaceutical composition of item 65, further comprising one or more additional antigen-binding protein(s) capable of binding to, blocking, or neutralizing one or more snake venom antigen(s).

67. A kit-of-parts comprising
    a) an antigen-binding protein according to anyone of items 1 to 61; and
    b) another agent suitable for the treatment of snake envenomation.

68. The kit-of-parts according to item 67, wherein said other agent used for the treatment of snake envenomation is one or more additional antigen-binding

protein(s) selected from the group consisting of: IgG, IgM, scFv, $F(ab)_2$, Fab, V_HH antigen-binding protein(s), and fragments thereof.

69. The kit-of-parts according to item 68, wherein the one or more additional antigen-binding protein(s) capable of neutralizing one or more snake venom antigen(s) is (are) selected from the group consisting of: Equine immunoglobulin(s) against snake venom, such as Equine IgG(s) or Equine Fab or Equine $F(ab')_2$ fragment(s) against snake venom, Ovine immunoglobulin(s) against snake venom, such as Ovine IgG(s) or Ovine Fab or Ovine $F(ab')_2$ fragment(s) against snake venom, Rabbit immunoglobulin(s) against snake venom, such as Rabbit IgG(s) or Rabbit Fab or Rabbits $F(ab')_2$ fragment(s) against snake venom, and Camelids immunoglobulin(s) against snake venom, such as Camelids IgG(s) or Camelids Fab or Camelids $F(ab')_2$ fragment(s) against snake venom.

70. The kit-of-parts according to item 67, wherein said other agent used for the treatment of snake envenomation is one or more protein(s) displaying neutralization potential against snake venoms.

71. The kit-of-parts according to item 70, wherein the one or more protein(s) displaying neutralization potential against snake venoms is (are) selected from the group consisting of : a PLA_2 inhibitor, a matrix metalloproteinase (MMP) inhibitor, a snake venom metalloproteinase inhibitor (SVMP), a Kunitz-type trypsin inhibitor, an antimyotoxic factor, an antimyonecrotic factor, an antineurotoxic factor, an antihæmmoragic factor, an antioedematogenic factor and a glycoprotein, such as acid-α1-glycoprotein.

72. The kit-of-parts according to item 67, wherein said other agent used for the treatment of snake envenomation is one or more small molecule(s) with inhibitory effect against snake toxins.

73. The kit-of-parts according to item 72, wherein the one or more small molecule(s) with inhibitory effect against snake toxins is (are) selected from the group consisting of: polyanions, polyphenolic compounds, and metalloproteinase inhibitors.

74. The kit-of-parts according to any one of items 72 and 73, wherein the one or more small molecule(s) with inhibitory effect against snake toxins is (are) selected from the list consisting of : Suramin, Heparin, Wedelolcatone, Coumestrol, Glycyrrhizin, Fucoidan, Apigenin analogue, AG-3340, Aristolochic acid, Rosmarinic acid, Imidazopyridine, BAPTA, TPEN, EDTA, DMPS, dimercaprol, BAY-129566, Marimastat, CGS-27023A, Batimastat, Nafamostat, MV 8612 from Mandevilla velutina, MV 8608 from Mandevilla velutina, Koninginin, 4-nerolidylcatechol, Anisodamine, 12-methoxy-4-methylvoachalotine (MMV), Quinonoid xanthene ehretianone, Benzoylsalireposide, Salireposide, Chlorogenic acid, Caffeic acid, 2-hydroxy-4-methoxy benzoic acid and, Varespladib.

75. An antigen-binding protein according to any one of items 1 to 61, a pharmaceutical composition according to any one of items 65 to 66 or a kit-of-parts according to item 67 to 74 for use in a method of treatment of snake envenomation in a subject.

76. The antigen-binding protein, the pharmaceutical composition or the kit-of-parts for the use according to item 75, wherein the antigen-binding protein, the pharmaceutical composition or the kit-of-parts prevents or delay venom-induced lethality.

77. The antigen-binding protein, the pharmaceutical composition or the kit-of-parts for the use according to any one of items 75 to 76, wherein the antigen-binding protein, the pharmaceutical composition or the kit-of-parts prevents or delays envenomation symptoms.

78. The antigen-binding protein, the pharmaceutical composition or the kit-of-parts for the use according to any one of items 75 to 77 wherein the antigen-binding protein delays venom-induced lethality for at least 8h, such as at least 10h, for instance at least 12h, such as at least 1 day, for instance at least 2 days, such as at least 3 days after envenomation.

79. The antigen-binding protein, the pharmaceutical composition or the kit-of-parts for the use according to any one of items 75 to 78, wherein the envenomation symptoms comprise limb paralysis, respiratory paralysis, redness, swelling, bruising, bleeding, blistering, severe pain at the site of the bite, tenderness at the site of the bite, nausea, vomiting, diarrhea, rapid heart rate, weak pulse, low blood pressure, disturbed vision, increased salivation, increased sweating, numbness, tingling and/or muscle twitching.

80. The antigen-binding protein, the pharmaceutical composition or the kit-of-parts for the use according to any one of items 75 to 79, wherein the antigen-binding protein, the pharmaceutical composition or the kit-of-parts is administered parenterally or pulmonarily.

81. The antigen-binding protein, the pharmaceutical composition or the kit-of-parts for the use according to item 80, wherein the parenteral administration of the antigen-binding protein, the pharmaceutical composition or the kit-of-parts is intravenous, intramuscular and/or sub-cutaneous.

82. The antigen-binding protein, the pharmaceutical composition or the kit-of-parts for the use according to any one of items 75 to 81, wherein the antigen-binding protein, the pharmaceutical composition or the kit-of-parts is administered no more than 5 min after envenomation, such as no more than 10 min after envenomation, for instance no more than 15 min after envenomation, such as no more than 20 min after envenomation, for instance no more than 30 min after envenomation, such as no more than 45 min after envenomation, for instance no more than 60 min after envenomation, such as no more than 90 min after envenomation, for instance no more than 120 min after envenomation, such as no more than 180 min after envenomation, for instance no more than 240 min after envenomation, such as no more than 6h after envenomation, for instance no more than 12h after envenomation, such as no more than 24h after envenomation, for instance no more than 48h after envenomation, such as no more than 72h after envenomation, for instance no more than 96h after envenomation, such as no more than 120h after envenomation for instance no more than 144h after envenomation.

83. A method of treating snake envenomation which comprises administering to a subject an effective amount of the antigen-binding protein according to any one of items 1 to 61, the pharmaceutical composition according to any one of items 65 to 66 or the kit-of-parts according to any one of items 67 to 74.

84. Use of the antigen-binding protein according to any one of items 1 to 61, the composition according to any one of items 65 to 66 or the kit-of-parts according to item 67 to 74 for the manufacture of a medicament for the treatment of snake envenomation.

85. A kit for detection and/or quantification of snake venom in a sample comprising:
- one or more antigen-binding proteins, such as two antigen-binding proteins, wherein at least one of said antigen-binding proteins is according to any one of items 1 to 61; and
- means for detection of a complex comprising said one or more antigen-binding proteins bound to snake venom.

86. The kit according to item 85, wherein the kit further comprises means for acquiring or isolating a sample from a subject.

87. The kit according to any one of items 85 to 86, wherein the sample is a blood sample, a urine sample, a smear of the bite site or a smear of the bite wound.

88. The kit according to item 87, wherein the blood sample is a plasma blood sample.

89. The kit according to any one items 85 to 88, wherein the kit is an immunoassay, such as a lateral flow assay, an ELISA, an electrochemical impedance spectroscopy-based assay, a Bilayer Interferometry (BLI)-based assay or a turbidimetric immunoassay.

90. An antigen-binding protein according to any one of items 1 to 61 for use in the diagnosis of snake envenomation in a subject.

91. Use of an antigen-binding protein according to any one of items 1 to 61 in an *in vitro* method for detection and/or diagnosis of snake envenomation in a subject.

92. The antigen-binding protein, the pharmaceutical composition or the kit-of-parts for the use according to any one of items 7575 to 82, the kit according to any of items 85 to 88, or the antigen-binding protein for use according to item 90 wherein the subject is a human.

93. The antigen-binding protein, the pharmaceutical composition or the kit-of-parts for the use according to any one of items 75 to 82, the kit according to any of items 85 to 88, or the antigen-binding protein for use according to item 90, wherein the subject is a non-human animal, wherein said non-human animal for example may belong to the family of Equidae, Bovidae, Camelidae, Canidae or Felidae.

94. The antigen-binding protein, the pharmaceutical composition, the kit-of-parts or the kit according to item 93, wherein the non-human animal is a horse, a cow, an ox, a camel, a dog or a cat.

95. A method for detecting snake venom in a sample, said method comprising:
- providing a sample for analysis;
- contacting the sample with one or more antigen-binding proteins as defined in any one of items 1 to 61 to said sample; and
- detecting a complex comprising said one or more antigen-binding proteins bound to a snake antigen.

96. The method according to item 95, wherein the method is an *in vitro* method.

97. The method according to any one of items 95 to 96, wherein the detection of the complex is performed by an immunoassay, such as a lateral flow assay, an ELISA, an electrochemical impedance spectroscopy-based assay, a Bilayer Interferometry (BLI)-based assay or a turbidimetric immunoassay.

98. The antigen-binding protein according to any one of items 1 to 64, wherein the AC-SINS shift of the antigen-binding protein is of no more than 11nm, for example no more than 15nm, such as no more than 20nm, for example no more than 25nm, such as no more than 30nm.

Examples

Example 1 : Affinity maturation, cross-panning selections, and scFv characterization

Material and methods :

Toxin preparation

α-cobratoxin (L8114), α-bungarotoxin (L8115), and whole venoms from *N. kaouthia* (L1323), *N. melanoleuca* (L1318), *D. polylepis* (L1309), and *O. hannah* (L1357) were obtained from Latoxan SAS, France. Venom fractions containing long α-neurotoxins (Dp7 from D. polylepis and Nm8 from *N. melanoleuca*) were isolated from crude venom by fractionation using RP-HPLC (Agilent 1200).

Venoms were fractionated using a C18 column (250 × 4.6 mm, 5 µm particle; Teknokroma) and elution was carried out at 1 mL/min using Solution A (water supplemented with 0.1% TFA) and a gradient towards solution B (acetonitrile supplemented with 0.1% TFA): 0% B for 5 min, 0–15% B over 10 min, 15–45% B over 60 min, 45–70% B over 10 min, and 70% B over 9 min (**Refs. 6 and 9**). Fractions were collected manually and evaporated using a vacuum centrifuge. Toxins were dissolved in phosphate buffered saline (PBS) and biotinylated using a 1:1 (toxin:biotinylation reagent) molar ratio for α-cobratoxin and 1:1.5 molar ratio for the remaining toxins as described elsewhere (**Ref. 2**). Following biotinylation, toxins were purified as well as the degree of biotinylation was determined as previously described (**Ref. 4**).

Library generation using chain-shuffling

Light chain-shuffled libraries containing the heavy chain of antibody 368_01_C05 were created as described previously (**Ref. 4**). The sequence of the variable region of the heavy chain of antibody 368_01_C05 is provided herein as SEQ ID NO: 61. Two libraries were created, one with kappa and one with lambda light chains. The size of the kappa library was 1.67×10^8 whereas the lambda library was 1.01×10^8.

Colony PCR revealed that 96% of the transformants to have successful heavy chain ligation.

Library rescue, solution-based phage display selection, and polyclonal DELFIA

Phages were rescued from the light chain-shuffled libraries and three rounds of selections were performed as described elsewhere (**Ref. 13**) with a few exceptions as outlined below.

Phages were not concentrated using PEG precipitation, but instead phage-containing supernatants were blocked in PBS supplemented with Milk Protein (MPBS) and used directly for selections. Additionally, deselection of streptavidin-specific phages was performed as described previously (**Ref. 4**).

Lastly, to obtain scFvs with high affinity and broad cross-reactivity to different long α-neurotoxins, cross-panning between α-cobratoxin and Dp7 was performed as previously described (**Ref. 3**) as well as antigen concentrations were lowered between each round of selection starting at 10 nM and ending at 4 pM (Fig. 1A). The kappa and lambda libraries were mixed before the first round of selections. To assess the polyclonal output of the selections a polyclonal DELFIA was performed determining binding to both α-cobratoxin, Dp7, and MPBS.

Subcloning, screening, and sequencing of scFvs

The genes encoding the scFvs from five of the obtained selection outputs (representing different cross-panning strategies) were subcloned into the pSANG10-3F expression vector to allow for monoclonal screening of the clones as described elsewhere (**Ref. 2**). Briefly, scFv-encoding genes from five selected output phage libraries were amplified using primers M13leadseq (AAATTATTATTCGCAATTCCTTTGGTTGTTCCT) and Notmycseq (GGCCCCATTCAGATCCTCTTCTGAGATGAG) before restriction using NcoI and NotI restriction endonuclease sites. The genes were ligated into pSANG10-3F and transformed into E. coli strain BL21 (DE3) (New England Biolabs). From each selection output 184 clones were selected and expressed in 96-well plates.

The scFvs were evaluated for their binding to α-cobratoxin, Dp7, streptavidin, and milk protein using a monoclonal DELFIA assay as described previously (**Ref. 4**). From this, 329 clones were cherry-picked and sequenced (Eurofins Genomics sequencing service) using S10b primer (GGCTTTGTTAGCAGCCGGATCTCA). The antibody frameworks and the CDR regions of the light chains were annotated using Geneious

Biologics (Biomatters), and 67 clones were identified as unique based on light chain CDR3 regions.

Reformatting to IgG and Fab format

A total of 62 clones were selected for reformatting into the fully human IgG1 and Fab format.

The reformatting into the IgG1 format was completed as described in Laustsen et al. (**Ref. 2**), whereas reformatting into the Fab format was completed as described in Ledsgaard et al. (**Ref. 4**).

The binding of the IgGs to α-cobratoxin, Dp7, Nm8, and streptavidin was assessed and ranked using an expression-normalized capture (ENC) assay described previously (**Ref. 4**).

Developability characterization

To aid in the selection of the top antibody candidates for further characterization, the biophysical behavior of the 62 reformatted clones in the IgG format was characterized using HPLC-SEC and AC-SINS. For HPLC-SEC, the purified antibodies were loaded onto a Superdex 200 Increase 5/150 column at a flow rate of 0.25 mL/min using an Agilent 1100 HPLC instrument. AC-SINS was performed as described in Liu et al. (**Ref. 14**) with the modifications described in Dyson et al. (**Ref. 10**).

Selection, expression, and purification of IgGs

Based on ranking and cross-reactivity in IgG binding in ENC assay, IgG expression yield, HPLC-SEC profile, AS-SINS shift, and sequence diversity six antibodies (2551_01_A12, 2551_01_B11, 2554_01_D11, 2555_01_A01, 2555_01_A04, and 2558_02_G09) were selected for further characterization. The IgGs were expressed and purified as previously described (**Ref. 2**).

Surface plasmon resonance

The binding affinity of the corresponding Fab versions of the top six affinity matured antibodies as well as the parental clone to α-cobratoxin and Dp7 was determined using Surface Plasmon Resonance (SPR) (BIAcore T100, GE Healthcare).

Antigen immobilization and affinity measurements were performed as previously described (**Ref. 4**).

Based on affinity measurements the top two clones for further characterization were selected to be 2551_01_A12 and 2554_01_D11.

Results :

Human light-chain-shuffled scFv-based phage display libraries were created by paring the heavy chain of antibody 368_01_C05 with a naïve repertoire of human light chains.

Then, phage display cross-panning selections using two toxins with 70% sequence identity, α-cobratoxin from *N. kaouthia* and α-elapitoxin from *D. polylepis*, were conducted according to the overview provided in Fig. 1A. Phage display selection outputs from the third round were subcloned into the pSANG10-3F expression vector, and 736 monoclonal scFvs were tested for their ability to bind to α-cobratoxin, α-elapitoxin, and streptavidin in both direct dissociation-enhanced lanthanide fluorescence immunoassays (DELFIAs) and expression-normalized capture (ENC) DELFIAs.

From here, 203 scFvs (all displaying binding to at least one of the two toxins with negligible binding to streptavidin) were randomly selected for sequencing.
Out of these, 67 scFvs were unique according to the sequence of their light chain CDR3 region, 2 of them having kappa light chains and the remaining 65 having lambda light chains.

The top 62 clones, based on sequence diversity and binding behavior, were reformatted to the fully human IgG1 format.

Following expression in HEK293 cells, ENC DELFIAs were run using the crude expression supernatant to rank the IgG binding to α-cobratoxin, α-elapitoxin, a venom fraction from *N. melanoleuca* (Nm8) containing a long neurotoxin homologous to OH-55 (Q53B58) and long neurotoxin 2 (P01388) (**Ref. 9**), as well as streptavidin.
This revealed that more than half of the clones were cross-reactive against all three toxins/venom fractions, demonstrating significant improvement in both binding and cross-reactivity when compared to the parental antibody.
To help guide the selection of lead candidates, the suitability of the 62 clones for future antibody development was investigated by characterizing biophysical properties that are indicative of developability of the antibody, e.g. indicate of how well the antibodies

can be developed and manufactured in large scale without aggregating, precipitating, denaturing, or having suboptimal pharmacokinetics *in vivo*. To this purpose, we analyzed the purity and non-specific column interaction pattern of all IgGs using size-exclusion chromatography (SEC). In addition, an AC-SINS assay (**Ref. 10**) was employed to asses propensity for self-aggregation. For this analysis, we also included an IgG from a previous study (2552_02_B02) (**Ref. 4**), that had been reported to neutralize lethality induced by N. kaouthia whole venom in vivo, but had never been characterized for cross-reactivity to other long chain α-neurotoxins nor been analyzed for its "developability" properties.

The SEC data (% monomeric content and relative elution volumes - a metric for assessing non-specific interaction with the SEC column), AC-SINS shifts, binding data, and expression yields (full dataset see Table 1) were used to select the top six antibody candidates for further characterization.

These antibodies were named as follows: 2551_01_A12, 2551_01_B11, 2554_01_D11, 2555_01_A01, 2555_01_A04, and 2558_02_G09 (Fig. 1B and Table 2). Additionally, the data showed that the previously published IgG 2552_02_B02 had an extremely poor developability score, both judged by its late elution in the SEC analysis and its high shift in the AC-SINS assay. In fact, this antibody performed at a similarly poor level as the 'poor developability' control (bococizumab, AC-SINS shift of 33 nm) that was used for comparison, whereas the antibodies derived from the 368_01_C05 parental clone all possessed developability profiles similar to the 'good developability' control antibody (Aliricumab, AC-SINS shift of 3 nm). In addition, 2552_02_B02 showed no cross-reactivity to any of the long chain α-neurotoxins it was tested against, clearly distinguishing its binding profile from the antibodies derived from the 368_01_C05 parent (Fig. 1B).

Table 1. Expression and developability data for 61 IgGs and parental IgG. The expression yield is provided for cultures in 96-well format. AC-SINS shift reflects IgG self-association propensity and SEC analysis provides information on elution volume as well as percentage of IgG monomer and dimers detected. In general an AC-SINS shift of less than 11 nm is preferred.

	Production	AC-SINS	SEC analysis		
Antibody ID	Yield (mg/L)	Shift (nm)	Elution (mL)	Monomer (%)	Dimer (%)
2551_01_A12	7,7	3	1,48	100,0	0,0
2554_01_D11	14,0	1	1,48	94,8	5,2
2552_02_B07	10,7	1	1,47	97,1	2,9
2558_02_G09	21,7	1	1,48	95,6	4,4
2551_01_A11	10,6	2	1,50	95,2	4,8
2554_01_E01	15,5	2	1,49	96,6	3,4
2551_01_B11	11,1	1	1,50	96,2	3,8
2554_02_F10	23,6	1	1,48	96,5	3,5
2551_01_A02	11,3	1	1,46	97,0	3,0
2552_01_G02	11,7	2	1,49	96,6	3,4
2554_01_E10	12,2	1	1,47	95,2	4,8
2554_02_F11	13,9	1	1,48	94,4	5,6
2555_01_A04	8,7	1	1,47	100,0	0,0
2555_01_A01	16,0	1	1,47	96,7	3,3
2554_02_G09	14,8	4	1,48	97,0	3,0
2558_01_E06	15,2	1	1,47	96,3	3,7
2554_01_E03	14,5	1	1,48	96,6	3,4
2554_01_C11	14,4	1	1,47	97,2	2,8
2554_01_D10	13,2	1	1,47	96,8	3,2
2554_01_E05	9,8	2	1,46	96,9	3,1
2555_02_D09	14,8	2	1,46	97,0	3,0
2555_02_D02	7,3	1	1,49	100,0	0,0
2555_01_B09	16,5	1	1,47	96,0	4,0
2554_01_D05	13,9	2	1,47	97,1	2,9
2554_01_C07	14,0	2	1,47	96,6	3,4
2558_01_E08	13,1	1	1,47	96,7	3,3
2554_02_G10	13,6	1	1,47	96,9	3,1

2558_02_F12	12,9	1	1,47	96,6	3,4
2555_02_D05	11,4	2	1,47	100,0	0,0
2551_02_E01	11,3	2	1,52	95,2	4,8
2558_02_H05	10,0	2	1,49	96,6	3,4
2558_02_G01	8,0	3	1,48	100,0	0,0
2558_02_G10	17,2	2	1,48	96,4	3,6
2551_01_B01	12,2	1	1,43	96,5	3,5
2554_02_H01	9,1	1	1,48	96,5	3,5
2554_02_G12	13,9	2	1,56	100,0	0,0
2554_01_D01	9,9	2	1,51	97,1	2,9
2551_01_A01	2,7	7	1,49	100,0	0,0
2554_02_G07	15,9	1	1,48	96,6	3,4
2554_02_F12	11,4	1	1,47	95,3	4,7
2555_01_B01	19,7	1	1,48	95,4	4,6
2558_01_E04	12,1	1	1,51	95,6	4,4
2558_02_H06	9,3	1	1,47	100,0	0,0
2558_01_E03	20,1	1	1,47	96,1	3,9
2558_01_D11	15,4	1	1,47	97,2	2,8
2558_02_G07	17,9	0	1,46	96,4	3,6
2555_01_B04	17,3	1	1,48	97,4	2,6
2558_02_G02	20,1	-1	1,48	96,2	3,8
2558_01_E02	10,7	2	1,48	100,0	0,0
2555_02_D06	21,4	0	1,46	96,9	3,1
2554_01_D09	7,3	2	1,46	100,0	0,0
2555_02_D07	17,2	0	1,48	96,2	3,8
2558_01_D12	15,6	1	1,47	95,9	4,1
2558_02_G03	14,4	1	1,49	91,1	8,9
2558_01_E10	16,7	0	1,48	96,6	3,4
2558_02_G04	17,6	0	1,46	97,3	2,7

2554_01_D04	3,8	3	1,47	100,0	0,0
2554_02_F04	18,9	-	1,49	95,5	4,5
2555_02_C08	16,2	-	1,55	97,2	2,8
2558_02_G05	15,1	-	1,47	97,0	3,0
2555_01_A08	12,3	-	1,48	96,1	3,9
2558_02_F10	6,9	-	1,47	100,0	0,0
368_01_C05	14,7	0	1,45	97,5	2,5

Table 2. AC-SINS shift, SEC analysis results (% monomer content and relative elution volume), and transient expression yields for the top six chain-shuffled IgGs (2551_01_A12, 2551_01_B11, 2554_01_D11, 2555_01_A01, 2555_01_A05, and 2558_02_G09) in comparison with the parental IgG (368_01_C05). IgG 2552_02_B02 from a previous study was also included.

	AC-SINS	SEC analysis		Production
Antibody ID	Shift (nm)	Monomer (%)	Elution (mL)	Yield (mg/L)
2551_01_A12	3	100.0	1.48	18.1
2554_01_D11	1	94.8	1.48	47.1
2558_02_G09	1	95.6	1.48	38.5
2551_01_B11	1	96.2	1.50	30.4
2555_01_A04	1	100.0	1.47	25.8
2555_01_A01	1	96.7	1.47	45.4
368_01_C05	0	97.5	1.45	30.0
2552_02_B02	32	100.0	1.82	22.6

Analysis of the antibody sequences revealed that the six affinity matured antibodies had light chains belonging to two different germlines, germline IGLV3-21 for 2551_01_B11, 2555_01_A01, 2555_01_A04, and 2558_02_G09 and germline IGLV6-57 for 2551_01_A12 and 2554_01_D11. The parental antibody had germline IGLV6-57, meaning that two of the six affinity matured antibodies had light chains belonging to

the same germline as the parental antibody. From the comparison of the three light chain CDR regions of the antibodies presented in Fig. 1C, it could also be seen that for the two antibodies maintaining the parental germline, the CDR-L2 was identical to the parental, whereas the CDR-L1 and CDR-L3 had 2-3 amino acid differences. For the remaining four antibodies with different light chain germline, all VL CDR sequences were significantly different from the parent antibody sequence.

To evaluate if the light-chain-shuffling campaign generated antibodies with improved affinity, surface plasmon resonance (SPR) was used to determine the affinity of the top six antibodies as well as the parental antibody. To this purpose, all antibodies were reformatted to the monovalent Fab format to measure 1:1 binding kinetics of each antibody against both α-cobratoxin and α-elapitoxin (for SPR sensograms see Fig.2.A and Fig.2.B for α-cobratoxin and α-elapitoxin respectively). Data showed that all six antibodies displayed higher affinity to both toxins as compared to the parental antibody (Table 3). The largest improvement was observed for 2551_01_A12 and 2554_01_D11 (32 and 50-fold improvement of binding to α-cobratoxin and 13 and 8-fold improvement of binding to α-elapitoxin, respectively), providing both antibodies with low single-digit nanomolar affinities to both toxins.
Thus, a significant improvement in both affinity and cross-reactivity was observed.

Antibodies 2551_01_A12 and 2554_01_D11 were selected for further characterization based on affinity, cross-reactivity, expression yield, and developability data.

Table 3. Affinity measurements using Surface Plasmon Resonance (SPR).
SPR was used to measure the affinity of the top six chain-shuffled antibodies (2551_01_A12, 2551_01_B11, 2554_01_D11, 2555_01_A01, 2555_01_A05, and 2558_02_G09) and the parental antibody (368_01_C05) in the Fab format to both α-cobratoxin and α-elapitoxin. The dissociation constants, on-rates, and off-rates are provided. For sensograms see Fig. 2.

	α-cobratoxin			α-elapitoxin		
Antibody ID	K_D (nM)	k_{on} (s^{-1})	k_{off} (M·s)	K_D (nM)	k_{on} (s^{-1})	k_{off} (M·s)
2551_01_A12	2.79	$5.02 \cdot 10^{-4}$	$1.80 \cdot 10^{5}$	1.12	$1.40 \cdot 10^{-4}$	$1.25 \cdot 10^{5}$
2554_01_D11	1.78	$2.29 \cdot 10^{-4}$	$1.28 \cdot 10^{5}$	1.69	$1.77 \cdot 10^{-4}$	$1.05 \cdot 10^{5}$
2558_02_G09	2.77	$5.94 \cdot 10^{-4}$	$2.14 \cdot 10^{5}$	3.04	$4.22 \cdot 10^{-4}$	$1.39 \cdot 10^{5}$
2551_01_B11	4.27	$5.00 \cdot 10^{-4}$	$1.17 \cdot 10^{5}$	2.87	$1.83 \cdot 10^{-4}$	$6.37 \cdot 10^{5}$
2555_01_A04	7.46	$1.65 \cdot 10^{-3}$	$2.22 \cdot 10^{5}$	2.21	$2.05 \cdot 10^{-4}$	$9.26 \cdot 10^{5}$
2555_01_A01	8.41	$1.70 \cdot 10^{-3}$	$2.02 \cdot 10^{5}$	1.81	$1.81 \cdot 10^{-4}$	$1.00 \cdot 10^{5}$
368_01_C05	89.6	$1.64 \cdot 10^{-2}$	$1.83 \cdot 10^{4}$	14.3	$9.25 \cdot 10^{-4}$	$6.47 \cdot 10^{4}$

Because the binding profiles of the cross-reactive antibodies derived from antibody 368_01_C05 were significantly different from antibody 2552_02_B02, SPR was used to determine if the antibodies bound the same or overlapping epitopes on α-cobratoxin. Using 2554_01_D11 as a representative of the cross-reactive antibodies, this study revealed that neither of the two antibodies 2552_02_B02 or 2554_01_D11 could bind α-cobratoxin if the other antibody was already bound to the toxin, suggesting that the antibodies recognized the same or overlapping epitopes.

Conclusion :

Antibodies with higher affinity to both α-cobratoxin and α-elapitoxin as compared to the parental antibody could be generated by the light-chain-shuffling method described hereinabove. Antibody candidates were selected for further development based on affinity, cross-reactivity, expression yield, and developability data.

Example 2 : Native mass spectrometry reveals cross-reactivity to several toxins from elapid snakes of three different genera.

Material and methods :

Determining cross-reactivity using native mass spectrometry

Sample preparation

Venoms and antibody samples were fractionated and exchanged into 200 mM ammonium acetate by size exclusion chromatography (SEC) as previously described

(**Refs. 15 and 16**). These experiments were performed on a Superdex Increase 200 10/300 GL column (Cytiva, Massachusetts, United States) pre-equilibrated with 200 mM ammonium acetate. Samples were collected and stored a 4 ºC until used. Prior to analysis, aliquots of the venom and IgG 2554_01_D11 SEC fractions were mixed in a 1:1 ratio (v/v). The final concentration of the antibody was approximately 3 µM after mixing. The concentration of toxins in the SEC fractions were not adjusted prior to mixing with the antibody.

Native mass spectrometry

All mass spectrometry (MS) experiments were performed on a SELECT SERIES cyclic IMS mass spectrometer (Waters, Manchester, U.K.) which was fitted with a 32,000 m/z quadrupole, as well as an electron capture dissociation (ECD) cell (MSvisions, Almere, Netherlands), the latter of which was situated in the transfer region of this mass spectrometer. Approximately 4 µL of sample were nano-sprayed from borosilicate capillaries (prepared in-house) fitted with a platinum wire. Spectra were acquired in a positive mode, with the m/z range set to 50-8,000. Acquisitions were performed for five minutes at a rate of 1 scan per second. The operating parameters for the MS experiments were as follows, unless otherwise stated: capillary voltage, 1.2 - 1.5 kV; sampling cone, 20 V; source offset, 30 V; source temperature, 28 °C; trap collision energy, 5 V; transfer collision energy, 5 V; Ion guide RF, 700 V. This instrument was calibrated with a 50:50 acetonitrile:water solution containing 20 µM cesium iodide (99.999%, analytical standard for HR-MS, Fluka, Buchs, Switzerland) each day prior to measurements.

Top-down proteomics of toxins bound by 2554_01_D11

The toxin:antibody complexes were purified using SEC, using the methods described above. Toxins were ejected from the protein complex during the MS experiments by setting the cone voltage to 160 V. The 5+ ions (most abundant charge state) of the ejected toxins were selected by tandem MS (MS/MS) and subjected to fragmentation by applying a trap voltage between 80 and 100 V as well as a transfer voltage between 20 and 50 V. Peptide sequence assignment was performed for 1+ fragmentation ions using the BioLynx package, which is a part of the MassLynx v4.1 software.

Sequence alignment

Sequence alignment was performed in Clustal Omega (**Ref. 17**) and visualised in Jalview (**Ref. 18**) using α-cobratoxin (P01391) from N. kaouthia, α-elapitoxin (P01396) from D. polylepis, α-bungarotoxin (P60615) from B. multicinctus, long neurotoxin 2 (A8N285) from O. hannah, and long neurotoxin (P0DQQ2) and long neurotoxin 2 (P01388) from N. melanoleuca.

Structures for each toxin were retrieved prioritising high-resolution X-ray resolved structures and included the following: P01391 = 1CTX (2.8Å, X-ray), P01388 = AF-P01388-F1 (AlphaFold2 predicted), P01396 = AF-P01396-F1 (AlphaFold2 predicted), P60615 = 1HC9 (1.8Å, X-ray), A8N285 = AF-A8N285-F1 (AlphaFold2 predicted), and P0DQQ2 = AF-P0DQQ2-F1 (AlphaFold2 predicted).

Structural alignment and root-mean-square deviation (RMSD) analysis were performed in ChimeraX (**Ref. 19**).

Epitopes of P01388 and P01396 were identified using the STAB Profiles tool (**Ref. 11**) (https://venom.shinyapps.io/stab_profiles/).

Results :

To further explore the cross-reactivity of the discovered antibodies, IgG 2554_01_D11 was tested for its binding to toxins in five whole venoms including *N. kaouthia, N. melanoleuca, N. naja, Ophiophagus hannah*, and *D. polylepsis*.

These venoms from African (*D. polylepsis* and *N. melanoleuca*) and Asian (*N. kaouthia, N. naja* and *O. hannah*) snakes all possess a relatively high content of long chain α-neurotoxins, ranging from 13.2% for *D. polylepsis* (**Ref. 6**) to 55% for *N. kaouthia* (**Ref. 5**), except *N. naja* that has been reported to have a long chain α-neurotoxin-content of about 2-5.

For this purpose, native mass spectrometry (MS), was used to investigate the interactions between the antibody and toxins from the four snake venoms.

Prior to native mass spectrometry analysis, the venoms and the IgG were fractionated using SEC (Fig. 3). The IgG was mixed with each of the SEC-generated toxin fractions, before analysis using native MS to determine binding (Fig. 3).

This analysis revealed that 2554_01_D11 only bound toxins of masses in the range expected for the group of three-finger toxins (3FTx) to which all α-neurotoxins belong.

To identify the toxins, the toxin:antibody complexes were isolated using MS/MS and subjected to collisional dissociation to eject the toxins from the antibody, allowing their intact mass to be determined. The primary dissociation product from these experiments were proteins of masses between 7,800 and 8,200 Da, corresponding to typical masses of long chain α-neurotoxins (Fig. 4).

The sequences of the toxins bound by 2554_01_D11 were investigated via top-down proteomics to confirm the identities of these toxins. For these experiments, the toxin:antibody complexes were purified using SEC. Toxins were dissociated from the antibody by applying a high cone voltage. This is a focusing voltage applied to the cone, which is located in the source region of the instrument. Increasing this voltage leads to harsh condition that can dissociate noncovalent complexes. Since this dissociation occurs before the quadrupole, the most prominent charge state of each ejected toxin could then be isolated using MS/MS for top-down sequencing. This isolation is important, as it ensures that the peptide fragmentation peaks only correspond to the toxin of interest. For the toxins of masses between 7,800 and 8,200 Da, only one readily discernible peptide fragment series was detected for each precursor ion. The limited amount of sequence data obtained from these experiments is attributed to the presence of disulfide bonds present in snake venom toxins, which cannot be broken using this fragmentation technique.

A BLAST search against all available elapid protein sequences revealed that the peptide sequences obtained by top-down analysis were unique to long chain α-neurotoxins and that each peptide only had one complete match to long chain α-neurotoxins homologs from the investigated venom.

Sequence data combined with the detected masses of the toxins revealed that 2554_01_D11 was capable of binding to long chain α-neurotoxin-containing SEC fractions across all tested venoms. This suggested that this antibody is cross-reactive against long chain α-neurotoxins present in all five tested venoms, further highlighting the broadly cross-reactive behavior of 2554_01_D11.

The toxin homologs specifically identified to be bound by 2554_01_D11 were long neurotoxin 2 (A8N285) from O. hannah, α-cobratoxin (P01391) from *N. kaouthia*, long neurotoxin 2 (P01388) and long neurotoxin (P0DQQ2) from *N. melanoleuca*, long

neurotoxin 4 (P25672) from *N. naja*, and α-elapitoxin (P01396) from *D. polylepis*. In addition, the antibody was shown to bind α-bungarotoxin (P60615) from *B. multicinctus* using SPR.

The average sequence similarity of the seven toxins was 62% (stdev: 9.9%), with an identity of 38% across all toxins; a total of 28 amino acid positions were identical across all toxins (Fig. 5A). The highest identity was observed between α-cobratoxin and long neurotoxin 2 from *N. melanoleuca* (83%) and the lowest identity was observed between long neurotoxin 2 from *N. melanoleuca* and α-bungarotoxin (51%). Additionally, a structural comparison was performed via root-mean-square deviation (RMSD) and revealed a mean pruned/total similarity of 0.81Å/3.1Å (stdev: 0.26Å/1.23Å), respectively; the best match appeared to be between long neurotoxin and long neurotoxin 2 from *N. melanoleuca* (0.23Å/0.23Å) and the worst match appeared to be between long neurotoxin 2 from *O. hannah* and α-cobratoxin (pruned: 1.18Å) and α-elapitoxin and α-bungarotoxin (total: 4.5Å; Fig 5B).
For α-cobratoxin, the amino acid residues involved in binding to the nicotinic acetylcholine receptor have been highlighted both in the sequence (Fig. 5A) and in the structure on the toxin (Fig. 5C).
Additionally, the residues that through a high-density peptide microarray-based study (**Ref. 11**) have been identified to be involved in the binding between antivenom-derived antibodies and α-elapitoxin and long neurotoxin 2 from *N. melanoleuca*, respectively, have been highlighted in the toxin sequence in Fig. 5A and in the toxin structure in Fig. 5D and 5E.

Conclusion :
The results indicate that the candidate antibody 2554_01_D11 is cross-reactive against a broad range long chain α-neurotoxins from at least 3 different elapid snakes genera.

Example 3 : Increased *in vitro* neutralization potency and broadening of cross-neutralization

Material and methods :

In vitro neutralization using electrophysiology (QPatch)

To determine the ability and potency with which the affinity matured clones 2551_01_A12 and 2554_01_D11, as well as the parental antibody 368_01_C05, were

able to neutralize the effects of α-cobratoxin, whole-cell patch-clamp experiments were conducted using Rhabdomyosarcoma cells (ACTT) as previously described (**Ref. 4**). In brief, the nAChR-mediated current elicited by acetylcholine was determined in the presence of 4 nM α-cobratoxin or 4 nM α-cobratoxin preincubated with different concentrations of the three IgGs on a QPatch II automated electrophysiology platform (Sophion Bioscience). As a control a dendrotoxin-specific IgG was included. The inhibitory effect of α-cobratoxin was normalized to the full acetylcholine response and a non-cumulative concentration-response plot was plotted. A Hill fit was used to obtain IC50 values for each of the three IgGs.

In vitro cross-neutralization using electrophysiology (Qube 384)

To determine the broader cross-neutralizing potential of the top two affinity matured antibodies and the parent antibody, automated patch-clamp experiments using the Qube 384 electrophysiology platform (Sophion Bioscience) were conducted. The experiments were conducted as described in Wade et al., Quads. The three IgGs (32.5 nM) were tested against α-cobratoxin (1.47 nM), α-bungarotoxin (6.5 nM), Dp7 (0.81 nM), Nm8 (14 nM), Nm3 (10.3 nM). A dendrotoxin-specific IgG was included as a control.

Results :

After having established the broadly cross-reactive nature of one of the top two chain-shuffled antibodies (2554_01_D11), automated patch-clamp technology was applied to assess if binding translated into functional neutralization in vitro for 2551_01_A12 and 2554_01_D11, as well as for the parental clone, 368_01_C05.

Here, a human derived cell line endogenously expressing the nAChR was used for measuring the acetylcholine-dependent current. α-cobratoxin inhibited this current in a concentration-dependent manner, and the IC80 value for the toxin was determined. Thereafter, the QPatch II system was used to determine the concentration-dependent neutralization of the current-inhibiting effect of α-cobratoxin by the three antibodies. Results demonstrated that all three antibodies were able to fully neutralize the effects of α-cobratoxin, whereas an irrelevant isotype control antibody (recognizing a dendrotoxin) had no effect (Fig. 6A).

The IC50 values were determined for the different antibodies: 368_01_C05 had an IC50 value of 4.9 nM (CI95 4.0-6.0 nM), 2551_01_A12 had an IC50 value of 2.6 nM

(CI95 2.2-3.2 nM), and 2554_01_D11 had an IC50 value of 1.7 nM (CI95 1.5-2.0 nM), thereby showing an increase in neutralizing potential following the chain-shuffling process.

These IC50 values translates into toxin:antibody molar ratios of 1:1.23 for 368_01_C05, 1:0.65 for 2551_01_A12, and 1:0.43 for 2554_01_D11. Since each IgG has two binding sites, the theoretically lowest amount of IgG needed to neutralize the effect of one toxin would be 0.5 IgGs.

To determine if the increased cross-reactivity to other α-neurotoxins translated into cross-neutralization, a single concentration antibody screen was set up using the Qube 384 system. Here, the three antibodies (368_01_C05, 2551_01_A12, and 2554_01_D11) were tested against α-cobratoxin from *N. kaouthia*, α-elapitoxin from *D. polylepis*, and Nm8 from *N. melanoleuca*, which all were toxins that 2554_01_D11 had been shown to bind through native MS. In addition, α-bungarotoxin was included, as it has 58% sequence identity to α-cobratoxin, is commercially available, and is an important toxin to neutralize in the venom of *B. multicinctus*. As a control, Nm3, a venom fraction from *N. melanoleuca* containing a short chain α-neurotoxin that also binds to the nAChR, but is not bound by any of the three antibodies, was included.

This automated patch-clamp screening revealed that α-cobratoxin and α-elapitoxin could be neutralized by all three antibodies in this assay (Fig. 6B).

Additionally, the chain-shuffled clones were able to neutralize α-bungarotoxin and partially neutralize the α-neurotoxins present Nm8, none of which was achieved by the parental clone.

Conclusion :

Collectively, the results of the *in vitro* neutralization assays using automated patch-clamp demonstrated that the chain-shuffled antibodies were both more potent in their neutralization of α-cobratoxin, as well as more broadly neutralizing than the parental antibody, inhibiting the effect of α-neurotoxins from snakes of three different genera inhabiting both Asia and Africa.

Based on binding, developability, affinity, expression, and in vitro neutralization data, 2554_01_D11 was selected as the top candidate for *in vivo* testing.

Example 4 : *In vitro* neutralization data translates to *in vivo* complete or partial neutralization of snake venoms from different genera and continents

Material and methods :

Animals

Animal experiments were conducted in CD-1 mice of both sexes weighing 18-20 g. Mice were supplied by Instituto Clodomiro Picado and experiments were conducted following protocols approved by the Institutional Committee for the Use and Care of Animals (CICUA), University of Costa Rica (approval number CICUA 82-08). Mice were provided food and water ad libitum and housed in cages in groups of 4.

In vivo preincubation experiments

The in vivo cross-neutralizing potential of 2554_01_D11 against whole venoms of *N. kaouthia, D. polylepis,* and *O. hannah* was assessed by i.v. injection of IgG preincubated with venom in groups of four mice.

Mixtures of a constant amount of venom and various amounts of antibody were prepared and incubated for 30 min at 37 °C. Then, aliquots of the mixtures, containing 2 LD50s of venoms (for *N. kaouthia* 9.12 µg, for *D. polylepis* 25.8 µg, and for *O. hannah* 40 µg) were injected in the caudal vein of mice using an injection volume of 150-200 µL. Control mice were injected with either venom alone, venom preincubated with an isotype control IgG or, in the cases of *N. kaouthia* and *D. polylepis* venoms, preincubated with commercial horse-derived antivenoms. For *N. kaouthia*, Snake Venom Antiserum from VINS Bioproducts Limited (Batch number: 01AS13100) was used at a ratio of 0.2 mg venom per mL antivenom. For D. polylepis, Premium Serum and Vaccines antivenom (Batch number: 062003) was used at a ratio of 0.12 mg venom/mL.

The IgG was injected using 1:1 and 1:2 α-neurotoxin:IgG molar ratio for *N. kaouthia* and *O. hannah* and 1:3 α-neurotoxin:IgG molar ratio for *D. polylepis*. For calculating molar ratios, based on toxicovenomic studies, it was estimated that 55% of *N. kaouthia* venom (**Ref. 5**), 13.2% of *D. polylepis* venom (**Ref. 6**), and 40% of *O. hannah* consisted of α-neurotoxins (**Ref. 20**).

Further, the *in vivo* neutralizing potential of antibody 2555_01_A01 against α-cbtx (α-cobratoxin) from the venom of *N. kaouthia* was assessed. The antibody was incubated with α-cbtx at toxin:antibody molar ratios of 1:1 and 1:2. Controls included α-cbtx incubated with either 0.12 M NaCl, 0.04 M phosphates, pH 7.2 (PBS, α-cbtx only) or with an isotype control IgG (Isotype IgG control). After incubation, aliquots of 100 µL, containing 4 µg α-cbtx, corresponding to 2 Median Lethal Doses (LD_{50}), were injected intravenously, in the caudal vein, to groups of four mice.Following injection, animals

were observed for signs of neurotoxicity, and survival was monitored for 48 hours. Results were presented in Kaplan-Meier plots.

In vivo rescue-type experiments

In order to assess whether the antibody was capable of neutralizing the venom of N. kaouthia in an experimental setting that more closely resembles the actual circumstances of envenoming, a rescue-type experiment was designed.
For this, the s.c. route was used for injection of venom, while the antibody was administered i.v.
First, the s.c. LD50 of *N. kaouthia* venom was estimated by injecting various doses of venom diluted in 100 µL of PBS into groups of four mice. Animals were observed during 24 hr, deaths were recorded and the LD50 was estimated by Probits.
For neutralization experiments, groups of four mice first received a challenge dose of venom by the s.c. route, corresponding to 2 LD50s. Then, either immediately or 10 min after envenoming, 100 µL of the 2554_01_D11 antibody were administered i.v. in the caudal vein.
Mice were observed for the onset of neurotoxic manifestations and times of death were recorded and presented in Kaplan-Meier plots.

Results :

To verify that the *in vitro* cross-neutralization potential of 2554_01_D11 translated into *in vivo* cross-neutralization, animal experiments were set up to evaluate the ability of the antibody to prevent or delay venom-induced lethality.
Here, snake venoms from three different species belonging to three genera, one from Africa, i.e. *D. polylepis*, and two from Asia, i.e. *N. kaouthia* and *O. hannah*, were included. Notably, each of these venoms contain a substantial amount of long chain α-neurotoxins (a relative abundance of 13.2% **(Ref. 6)**, 55% **(Ref. 5)**, and 40%, respectively). Two LD50s of each venom were preincubated with 2554_01_D11 in a 1:1 and 1:2 toxin:antibody molar ratio for *N. kaouthia* and *O. hannah* or a 1:3 toxin:antibody molar ratio for *D. polylepis* before being administered i.v. to the mice. As controls, mice were injected with venom alone, venom preincubated with commercial antivenoms (except in the case of *O. hannah*, where no antivenom was available), or venom preincubated with an antibody isotype control.

Results of the studies demonstrated that all mice in the venom only control group, as well as the mice receiving venom preincubated with the isotype control antibody, died within the first hour after the challenge, with evident signs of limb paralysis and respiratory difficulty.

As expected, mice receiving *N. kaouthia* or *D. polylepis* venoms preincubated with commercially available antivenoms survived for the entire observation period, and no signs of neurotoxicity were observed.

In experiments where mice were injected with venoms incubated with 2554_01_D11, results varied depending on the venom. In the case of *N. kaouthia* venom, complete neutralization was observed at both toxin:antibody molar ratios, since mice survived during the 48-hour observation time. Moreover, mice did not show any signs of neurotoxicity, i.e., limb paralysis or respiratory difficulty along the whole period. In the case of *O. hannah*, there was a dose-dependent delay in the time of death, as compared to controls receiving venom alone. Likewise, a delay in the time of death was observed in the case of *D. polylepis* venom (Fig. 7).

Further, in a similar experiment, 2555_01_A01 was also shown to prevent lethality in mice during the traditional 24-hour assay period, while mice control groups treated with α-cbtx pre-incubated with PBS or Isotype IgG died within 30min after injection (Fig. 8).

Next, the ability of the antibody to abrogate lethality of N. kaouthia venom in rescue-type experiments was assessed. For this, the subcutaneous (s.c.) route of venom injection was used to better reproduce the actual circumstances of envenoming. The estimated LD50 by the s.c. route was 10.3 µg (95% confidence interval: 5.0 – 16.8 µg). Mice were challenged by the s.c. route with a dose of venom corresponding to 2 LD50s, i.e., 20 µg, followed by the i.v. administration of the 2554_01_D11 antibody in a volume of 100 µL, containing 535 µg protein. Control mice injected with venom only died within 40 – 60 min, with evident signs of limb and respiratory paralysis. When the antibody was administered immediately after venom injection, all mice survived the 24-hour observation period and did not show any evidence of limb or respiratory paralysis. When the antibody was provided 10 min after venom injection, two out of four mice died, but there was a delay in the time of death (150 min and 180 min). The other two mice survived the 24-hour observation time and did not show signs of paralysis (Fig 7D).

Conclusion :

The antibody candidate 2554_01_D11 successfully delayed or abrogated death from envenomation from snake venoms of 3 different genera *in vivo*.

Sequence overview

Due to the design of the antigen-binding proteins of some embodiments of the present invention, the SEQ ID NOs: 1, 9, 17, 25, 33, 41, and 49 are identical. For similar reasons, the SEQ ID NOs: 2, 10, 18, 26, 34, 42, and 50 are identical. Finally, also for similar reasons, the SEQ ID NOs: 3, 11, 19, 27, 35, and 51 are identical.

SEQ ID NO: 1

Protein sequence of variable heavy chain CDR1 (CDR-H1) of antibody 368_01_C05

GGTFSSYA

SEQ ID NO: 2

Protein sequence of variable heavy chain CDR2 (CDR-H2) of antibody 368_01_C05

IIPIFGTA

SEQ ID NO: 3

Protein sequence of variable heavy chain CDR3 (CDR-H3) of antibody 368_01_C05

DNLGYCSGGSCYSDYYYYYMDV

SEQ ID NO: 4

Protein sequence of the variable light chain of antibody 368_01_C05

NFMLTQPHSVSESPGKTVTISCTRSSGSIASTYVHWYQQRPGSAPTTVIYEDNQRPS GVPDRFSGSIDSSSNSASLTISGLKTEDEADYYCQSYDSSNGSVVFGGGTKLTVL

SEQ ID NO: 5

Protein sequence of variable light chain CDR1 (CDR-L1) of antibody 368_01_C05

SGSIASTY

SEQ ID NO: 6

Protein sequence of variable light chain CDR2 (CDR-L2) of antibody 368_01_C05

EDN

SEQ ID NO: 7

Protein sequence of variable light chain CDR3 (CDR-L3) of antibody 368_01_C05

QSYDSSNGSVV

SEQ ID NO: 8

Protein sequence of the variable heavy chain of antibody 2551_01_A12
QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGGIIPIFGT
ANYAQKFQGRVTITADESTSTAYMELRSLRSDDTAVYYCARDNLGYCSGGSCYSDYY
YYYMDVWGQGTLVTVSS

SEQ ID NO: 9

Protein sequence of variable heavy chain CDR1 (CDR-H1) of antibody 2551_01_A12
GGTFSSYA

SEQ ID NO: 10

Protein sequence of variable heavy chain CDR2 (CDR-H2) of antibody 2551_01_A12
IIPIFGTA

SEQ ID NO: 11

Protein sequence of variable heavy chain CDR3 (CDR-H3) of antibody 2551_01_A12
DNLGYCSGGSCYSDYYYYYMDV

SEQ ID NO: 12

Protein sequence of the variable light chain of antibody 2551_01_A12
NFMLTQPHSVSESPGKTVTFSCTRSSGRIVSDYVHWYQQRPGSAPTTVIYEDNQRPS
GVPDRFSGSIDSSSNSASLTISGLKTEDEADYYCQSYDSSNAYVVFGGGTKVTVL

SEQ ID NO: 13

Protein sequence of variable light chain CDR1 (CDR-L1) of antibody 2551_01_A12
SGRIVSDY

SEQ ID NO: 14

Protein sequence of variable light chain CDR2 (CDR-L2) of antibody 2551_01_A12
EDN

SEQ ID NO: 15

Protein sequence of variable light chain CDR3 (CDR-L3) of antibody 2551_01_A12
QSYDSSNAYVV

SEQ ID NO: 16

Protein sequence of the variable heavy chain of antibody 2554_01_D11
QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGGIIPIFGT
ANYAQKFQGRVTITADESTSTAYMELRSLRSDDTAVYYCARDNLGYCSGGSCYSDYY
YYYMDVWGQGTLVTVSS

SEQ ID NO: 17

Protein sequence of variable heavy chain CDR1 (CDR-H1) of antibody 2554_01_D11
GGTFSSYA

SEQ ID NO: 18

Protein sequence of variable heavy chain CDR2 (CDR-H2) of antibody 2554_01_D11

IIPIFGTA

SEQ ID NO: 19

Protein sequence of variable heavy chain CDR3 (CDR-H3) of antibody 2554_01_D11

DNLGYCSGGSCYSDYYYYYMDV

SEQ ID NO: 20

Protein sequence of the variable light chain of antibody 2554_01_D11

NFMLTQPRSVSESPGKTVTISCTRSSGSIGSDYVHWYQQRPGSSPTTVIYEDNQRPS
GVPDRFSGSIDSSSNSASLTISGLKTEDEADYYCQSYDRSNHEVVFGGGTKLTVL

SEQ ID NO: 21

Protein sequence of variable light chain CDR1 (CDR-L1) of antibody 2554_01_D11

SGSIGSDY

SEQ ID NO: 22

Protein sequence of variable light chain CDR2 (CDR-L2) of antibody 2554_01_D11

EDN

SEQ ID NO: 23

Protein sequence of variable light chain CDR3 (CDR-L3) of antibody 2554_01_D11

QSYDRSNHEVV

SEQ ID NO: 24

Protein sequence of the variable heavy chain of antibody 2551_01_B11

QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGGIIPIFGT
ANYAQKFQGRVTITADESTSTAYMELRSLRSDDTAVYYCARDNLGYCSGGSCYSDYY
YYYMDVWGQGTLVTVSS

SEQ ID NO: 25

Protein sequence of variable heavy chain CDR1 (CDR-H1) of antibody 2551_01_B11

GGTFSSYA

SEQ ID NO: 26

Protein sequence of variable heavy chain CDR2 (CDR-H2) of antibody 2551_01_B11

IIPIFGTA

SEQ ID NO: 27

Protein sequence of variable heavy chain CDR3 (CDR-H3) of antibody 2551_01_B11

DNLGYCSGGSCYSDYYYYYMDV

SEQ ID NO: 28

Protein sequence of the variable light chain of antibody 2551_01_B11

SYELTQPPSVSVAPGQTATITCGGHNIGSNIVHWYHQKPGQAPELVISHNTNRPSGIP ERFSGSNSGSTATLTISRVEAVDEADYYCQVWDSSSEHVVFGGGTKVTVL

SEQ ID NO: 29

Protein sequence of variable light chain CDR1 (CDR-L1) of antibody 2551_01_B11

NIGSNI

SEQ ID NO: 30

Protein sequence of variable light chain CDR2 (CDR-L2) of antibody 2551_01_B11

HNT

SEQ ID NO: 31

Protein sequence of variable light chain CDR3 (CDR-L3) of antibody 2551_01_B11

QVWDSSSEHVV

SEQ ID NO: 32

Protein sequence of the variable heavy chain of antibody 2555_01_A01

QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGGIIPIFGT ANYAQKFQGRVTITADESTSTAYMELRSLRSDDTAVYYCARDNLGYCSGGSCYSDYY YYYMDVWGQGTLVTVSS

SEQ ID NO: 33

Protein sequence of variable heavy chain CDR1 (CDR-H1) of antibody 2555_01_A01

GGTFSSYA

SEQ ID NO: 34

Protein sequence of variable heavy chain CDR2 (CDR-H2) of antibody 2555_01_A01

IIPIFGTA

SEQ ID NO: 35

Protein sequence of variable heavy chain CDR3 (CDR-H3) of antibody 2555_01_A01

DNLGYCSGGSCYSDYYYYYMDV

SEQ ID NO: 36

Protein sequence of the variable light chain of antibody 2555_01_A01

SYELTQPPSVSVAPGRTATITCEGDNIGQQIVHWYQQKPGQAPVAVISSDSDRPSGIP ERFSGSNSGNTATLTISRVEAGDEADYYCQVWDSGSDHVVFGGGTKVTVL

SEQ ID NO: 37

Protein sequence of variable light chain CDR1 (CDR-L1) of antibody 2555_01_A01

NIGQQI

SEQ ID NO: 38

Protein sequence of variable light chain CDR2 (CDR-L2) of antibody 2555_01_A01

SDS

SEQ ID NO: 39

Protein sequence of variable light chain CDR3 (CDR-L3) of antibody 2555_01_A01

QVWDSGSDHVV

SEQ ID NO: 40

Protein sequence of the variable heavy chain of antibody 2555_01_A04

QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGGIIPIFGT ANYAQKFQGRVTITADESTSTAYMELRSLRSDDTAVYYCARDNLGYCSGGSCYSDYY YYYMDVWGQGTLVTVSS

SEQ ID NO: 41

Protein sequence of variable heavy chain CDR1 (CDR-H1) of antibody 2555_01_A04

GGTFSSYA

SEQ ID NO: 42

Protein sequence of variable heavy chain CDR2 (CDR-H2) of antibody 2555_01_A04

IIPIFGTA

SEQ ID NO: 43

Protein sequence of variable heavy chain CDR3 (CDR-H3) of antibody 2555_01_A04

DNLGYCSGGSCYSDYYYYYMDV

SEQ ID NO: 44

Protein sequence of the variable light chain of antibody 2555_01_A04

SYELTQPPSMSVAPGQTARITCGGDYIGGESVHWYQQKPGRAPVAVVYDDTHRPSG IPERFSGANSGNTATLTISKVEAGDEADYYCQVWDVSSDHVVFGGGTKLTVL

SEQ ID NO: 45

Protein sequence of variable light chain CDR1 (CDR-L1) of antibody 2555_01_A04

YIGGES

SEQ ID NO: 46

Protein sequence of variable light chain CDR2 (CDR-L2) of antibody 2555_01_A04

DDT

SEQ ID NO: 47

Protein sequence of variable light chain CDR3 (CDR-L3) of antibody 2555_01_A04

QVWDVSSDHVV

SEQ ID NO: 48

Protein sequence of the variable heavy chain of antibody 2558_02_G09
QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGGIIPIFGT
ANYAQKFQGRVTITADESTSTAYMELRSLRSDDTAVYYCARDNLGYCSGGSCYSDYY
YYYMDVWGQGTLVTVSS

SEQ ID NO: 49

Protein sequence of variable heavy chain CDR1 (CDR-H1) of antibody 2558_02_G09
GGTFSSYA

SEQ ID NO: 50

Protein sequence of variable heavy chain CDR2 (CDR-H2) of antibody 2558_02_G09
IIPIFGTA

SEQ ID NO: 51

Protein sequence of variable heavy chain CDR3 (CDR-H3) of antibody 2558_02_G09
DNLGYCSGGSCYSDYYYYYMDV

SEQ ID NO: 52

Protein sequence of the variable light chain of antibody 2558_02_G09
SYELTQPPSVSVAPGRTATITCEGDNIGQQIVHWYRQKSDQAPVVVVYDGSRRPSGI
PERFSGSNSGNTATLTISRVEAGDEADYYCQVWDITSDHVVFGGGTQLTVL

SEQ ID NO: 53

Protein sequence of variable light chain CDR1 (CDR-L1) of antibody 2558_02_G09
NIGQQI

SEQ ID NO: 54

Protein sequence of variable light chain CDR2 (CDR-L2) of antibody 2558_02_G09
DGS

SEQ ID NO: 55

Protein sequence of variable light chain CDR3 (CDR-L3) of antibody 2558_02_G09
QVWDITSDHVV

SEQ ID NO: 56

Protein sequence of the proposed identity of the *O. hannah* toxin identified bound by the antibody 2554_01_D11
TKCYVTPDATSQTCPDGENICYTKSWCDVFCSSRGKRIDLGCAATCPKVKPGVDIKC
CSTDNCNPFTPWKRH

SEQ ID NO: 57

Protein sequence of the proposed identity of the *N. naja* toxin identified bound by the antibody 2554_01_D11
IRCFITPDITSKDCPNGHVCYTKTWCDGFCRIRGERVDLGCAATCPTVKTGVDIQCCS
TDDCDPFPTRKRP

SEQ ID NO: 58

Protein sequence of the proposed identity of the *N. kaouthia* toxin identified bound by the antibody 2554_01_D11

IRCFITPDITSKDCPNGHVCYTKTWCDAFCSIRGKRVDLGCAATCPTVKTGVDIQCCST
DNCNPFPTRKRP

SEQ ID NO: 59

Protein sequence of the proposed identity of the *N. melanoleuca* toxin identified bound by the antibody 2554_01_D11

IRCFITPDVTSQICADGHVCYTKTWCDAWCTSRGKRVDLGCAATCPTVKTGVDIKCCS
TDNCNPFPTRNRP

SEQ ID NO: 60

Protein sequence of the proposed identity of the *D. polylepis* toxin identified bound by the antibody 2554_01_D11

RTCNKTFSDQSKICPPGENICYTKTWCDAWCSQRGKRVELGCAATCPKVKAGVEIKC
CSTDDCDKFQFGKPR

SEQ ID NO: 61

Protein sequence of the variable heavy chain of antibody 368_01_C05

QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGGIIPIFGT
ANYAQKFQGRVTITADESTSTAYMELRSLRSDDTAVYYCARDNLGYCSGGSCYSDYY
YYYMDVWGQGTLVTVSS

SEQ ID NO: 62

Polynucleotide sequence of M13leadseq primerAAATTATTATTCGCAATTCCTTTGGTTGTTCCT

SEQ ID NO: 63

Polynucleotide sequence of primer Notmycseq

GGCCCCATTCAGATCCTCTTCTGAGATGAG

SEQ ID NO: 64

Polynucleotide sequence of primer S10b

GGCTTTGTTAGCAGCCGGATCTCA

SEQ ID NO: 65

Protein sequence of the epitope region of the *O. hannah* toxin identified bound by the antibody 2554_01_D11

GCAATCPKVKPGVDIKCCST

SEQ ID NO: 66

Protein sequence of the epitope region of the *N. naja* and *N. kaouthia* toxins identified bound by the antibody 2554_01_D11

CAATCPTVKTGVDIQCCSTD

SEQ ID NO: 67

Protein sequence of the epitope region of the *N. melanoleuca* toxin identified bound by the antibody 2554_01_D11

CAATCPTVKTGVDIKCCSTD

SEQ ID NO: 68

Protein sequence of the epitope region of the *D. polylepis* toxin identified bound by the antibody 2554_01_D11

GCAATCPKVKAGVEIKCCST

SEQ ID NO: 69

Consensus sequence of the epitope bound by the antibody 2554_01_D11

CAATCPXVKXGVXIXCCST

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Claims

1. An antigen-binding protein capable of binding to, blocking or neutralizing two or more different long-chain α-neurotoxins, for example at least 3, such as at least 4, for example at least 5, such as at least 6, for example at least 7 different long-chain α-neurotoxins, wherein said antigen-binding protein binds to two or more different long-chain α-neurotoxins with a Kd of at the most 10nM.

2. The antigen-binding protein according to claim 1, wherein the long-chain α-neurotoxins are selected from the group consisting of: α-elapitoxins, α-bungarotoxins and α-cobratoxin, more preferably wherein at least one of said long-chain α-neurotoxins is an α-elapitoxin and /or at least one α-elapitoxin is α-elapitoxin-Dppd2, most preferably wherein the long-chain α-neurotoxins are selected from the group consisting of: long neurotoxin 2 (A8N285) from *O. hannah*, α-cobratoxin (P01391) from *N. kaouthia*, long neurotoxin 2 (P01388) and long neurotoxin (P0DQQ2) from *N. melanoleuca*, long neurotoxin 4 (P25672) from *N. naja*, and α-elapitoxin (P01396) from *D. polylepis*, preferably wherein the α-neurotoxins are selected from the group consisting of: a long neurotoxin 2 (P01388) from *N. melanoleuca* and a long neurotoxin homologous to OH-55 from *O. hannah*.

3. The antigen-binding protein according to any one of the preceding claims, wherein the long-chain α-neurotoxins are from one or more elapid snake(s), preferably wherein the elapid snake(s) is (are) from the genera *Aspidelaps, Boulengerina, Bungarus, Calliophis, Dendroaspis, Elapsoidea, Hemachatus, Hemibungarus, Micruroides, Micrurus, Naja, Ophiophagus, Paranaja, Pseudohaje* and/or *Walterinnesia, even more preferably* wherein the elapid snake(s) is (are) from the genera *Bungarus, Dendroaspis, Naja,* and/or *Ophiophagus*, most preferably wherein the elapid snake(s) is (are) *Naja kaouthia, Naja melanoleuca, Naja Naja, Dendroaspis polylepis, Ophiophagus Hannah* and/or *Bungarus multicinctus*.

4. The antigen-binding protein according to any one of the preceding claims capable of blocking or neutralizing the interaction between the nicotinic

acetylcholine receptor and two or more different long-chain α-neurotoxins and/or whole snake venom.

5. The antigen-binding protein according to any one of the preceding claims, wherein said antigen-binding protein comprises or consists of:

a) a heavy chain variable (VH) region comprising:

i. a heavy chain complementarity-determining region 1 (CDR-H1) comprising or consisting of SEQ ID NO: 17 or a variant thereof, wherein in said variant up to 3 amino acids have been altered , for example wherein 2 or 1 amino acid(s) have been altered ;

ii. a heavy chain complementarity-determining region 2 (CDR-H2) comprising or consisting of SEQ ID NO: 18 or a variant thereof, wherein in said variant up to 3 amino acids have been altered , for example wherein 2 or 1 amino acid(s) have been altered ; and

iii. a heavy chain complementarity-determining region 3 (CDR-H3) comprising or consisting of SEQ ID NO: 19 or a variant thereof, wherein in said variant up to 3 amino acids have been altered , for example wherein 2 or 1 amino acid(s) have been altered ; and

b) a light chain variable (VL) region comprising:

i. a light chain complementarity-determining region 1 (CDR-L1) comprising or consisting of SEQ ID NO: 21 or a variant thereof, wherein in said variant up to 3 amino acids have been altered , for example wherein 2 or 1 amino acid(s) have been altered ;

ii. a light chain complementarity-determining region 2 (CDR-L2) comprising or consisting of SEQ ID NO: 22 or a variant thereof, wherein in said variant up to 3 amino acids have been altered , for example wherein 2 or 1 amino acid(s) have been altered ; and

iii. a light chain complementarity-determining region 3 (CDR-L3) comprising or consisting of SEQ ID NO: 23 or a variant thereof, wherein in said variant up to 3 amino acids have been altered , for example wherein 2 or 1 amino acid(s) have been altered ;

or

a) a heavy chain variable (VH) region comprising:

i. a heavy chain complementarity-determining region 1 (CDR-H1) comprising or consisting of SEQ ID NO: 9 or a variant thereof, wherein in said variant up to 3 amino acids have been altered , for example wherein 2 or 1 amino acid(s) have been altered ;

ii. a heavy chain complementarity-determining region 2 (CDR-H2) comprising or consisting of SEQ ID NO: 10 or a variant thereof, wherein in said variant up to 3 amino acids have been altered , for example wherein 2 or 1 amino acids have been altered ; and

iii. a heavy chain complementarity-determining region 3 (CDR-H3) comprising or consisting of SEQ ID NO: 11 or a variant thereof, wherein in said variant up to 3 amino acids have been altered , for example wherein 2 or 1 amino acids have been altered ; and

b) a light chain variable (VL) region comprising:

i. a light chain complementarity-determining region 1 (CDR-L1) comprising or consisting of SEQ ID NO: 13 or a variant thereof, wherein in said variant up to 3 amino acids have been altered , for example wherein 2 or 1 amino acid(s) have been altered ;

ii. a light chain complementarity-determining region 2 (CDR-L2) comprising or consisting of SEQ ID NO: 14 or a variant thereof, wherein in said variant up to 3 amino acids have been altered , for example wherein 2 or 1 amino acid(s) have been altered ; and

iii. a light chain complementarity-determining region 3 (CDR-L3) comprising or consisting of SEQ ID NO: 15 or a variant thereof, wherein in said variant up to 3 amino acids have been altered , for example wherein 2 or 1 amino acid(s) have been altered ;

or

a) a heavy chain variable (VH) region comprising:
   i. a heavy chain complementarity-determining region 1 (CDR-H1) comprising or consisting of SEQ ID NO: 25 or a variant thereof, wherein in said variant up to 3 amino acids have been altered , for example wherein 2 or 1 amino acid(s) have been altered ;
   ii. a heavy chain complementarity-determining region 2 (CDR-H2) comprising or consisting of SEQ ID NO: 26 or a variant thereof, wherein in said variant up to 3 amino acids have been altered , for example wherein 2 or 1 amino acid(s) have been altered ; and
   iii. a heavy chain complementarity-determining region 3 (CDR-H3) comprising or consisting of SEQ ID NO: 27 or a variant thereof, wherein in said variant up to 3 amino acids have been altered , for example wherein 2 or 1 amino acids have been altered ; and
b) a light chain variable (VL) region comprising:
   i. a light chain complementarity-determining region 1 (CDR-L1) comprising or consisting of SEQ ID NO: 29 or a variant thereof, wherein in said variant up to 3 amino acids have been altered , for example wherein 2 or 1 amino acid(s) have been altered ;
   ii. a light chain complementarity-determining region 2 (CDR-L2) comprising or consisting of SEQ ID NO: 30 or a variant thereof, wherein in said variant up to 3 amino acids have been altered , for example wherein 2 or 1 amino acid(s) have been altered ; and
   iii. a light chain complementarity-determining region 3 (CDR-L3) comprising or consisting of SEQ ID NO: 31 or a variant thereof, wherein in said variant up to 3 amino acids have been altered , for example wherein 2 or 1 amino acid(s) have been altered ;

or

a) a heavy chain variable (VH) region comprising:
   i. a heavy chain complementarity-determining region 1 (CDR-H1) comprising or consisting of SEQ ID NO: 33 or a variant thereof,

wherein in said variant up to 3 amino acids have been altered , for example wherein 2 or 1 amino acid(s) have been altered ;

ii. a heavy chain complementarity-determining region 2 (CDR-H2) comprising or consisting of SEQ ID NO: 34 or a variant thereof, wherein in said variant up to 3 amino acids have been altered , for example wherein 2 or 1 amino acid(s) have been altered ; and

iii. a heavy chain complementarity-determining region 3 (CDR-H3) comprising or consisting of SEQ ID NO: 35 or a variant thereof, wherein in said variant up to 3 amino acids have been altered , for example wherein 2 or 1 amino acid(s) have been altered ; and

b) a light chain variable (VL) region comprising:

i. a light chain complementarity-determining region 1 (CDR-L1) comprising or consisting of SEQ ID NO: 37 or a variant thereof, wherein in said variant up to 3 amino acids have been altered , for example wherein 2 or 1 amino acid(s) have been altered ;

ii. a light chain complementarity-determining region 2 (CDR-L2) comprising or consisting of SEQ ID NO: 38 or a variant thereof, wherein in said variant up to 3 amino acids have been altered , for example wherein 2 or 1 amino acid(s) have been altered ; and

iii. a light chain complementarity-determining region 3 (CDR-L3) comprising or consisting of SEQ ID NO: 39 or a variant thereof, wherein in said variant up to 3 amino acids have been altered , for example wherein 2 or 1 amino acid(s) have been altered ;

or

a) a heavy chain variable (VH) region comprising:

i. a heavy chain complementarity-determining region 1 (CDR-H1) comprising or consisting of SEQ ID NO: 41 or a variant thereof, wherein in said variant up to 3 amino acids have been altered , for example wherein 2 or 1 amino acid(s) have been altered ;

ii. a heavy chain complementarity-determining region 2 (CDR-H2) comprising or consisting of SEQ ID NO: 42 or a variant thereof, wherein in said variant up to 3 amino acids have been altered , for example wherein 2 or 1 amino acid(s) have been altered ; and

iii. a heavy chain complementarity-determining region 3 (CDR-H3) comprising or consisting of SEQ ID NO: 43 or a variant thereof, wherein in said variant up to 3 amino acids have been altered , for example wherein 2 or 1 amino acid(s) have been altered ; and

b) a light chain variable (VL) region comprising:

i. a light chain complementarity-determining region 1 (CDR-L1) comprising or consisting of SEQ ID NO: 45 or a variant thereof, wherein in said variant up to 3 amino acids have been altered , for example wherein 2 or 1 amino acid(s) have been altered ;

ii. a light chain complementarity-determining region 2 (CDR-L2) comprising or consisting of SEQ ID NO: 46 or a variant thereof, wherein in said variant up to 3 amino acids have been altered , for example wherein 2 or 1 amino acid(s) have been altered ; and

iii. a light chain complementarity-determining region 3 (CDR-L3) comprising or consisting of SEQ ID NO: 47 or a variant thereof, wherein in said variant up to 3 amino acids have been altered , for example wherein 2 or 1 amino acid(s) have been altered ;

or

a) a heavy chain variable (VH) region comprising:

i. a heavy chain complementarity-determining region 1 (CDR-H1) comprising or consisting of SEQ ID NO: 49 or a variant thereof, wherein in said variant up to 3 amino acids have been altered , for example wherein 2 or 1 amino acid(s) have been altered ;

ii. a heavy chain complementarity-determining region 2 (CDR-H2) comprising or consisting of SEQ ID NO: 50 or a variant thereof, wherein in said variant up to 3 amino acids have been altered ,

for example wherein 2 or 1 amino acid(s) have been altered ; and

iii. a heavy chain complementarity-determining region 3 (CDR-H3) comprising or consisting of SEQ ID NO: 51 or a variant thereof, wherein in said variant up to 3 amino acids have been altered , for example wherein 2 or 1 amino acid(s) have been altered ; and

b) a light chain variable (VL) region comprising:

i. a light chain complementarity-determining region 1 (CDR-L1) comprising or consisting of SEQ ID NO: 53 or a variant thereof, wherein in said variant up to 3 amino acids have been altered , for example wherein 2 or 1 amino acid(s) have been altered ;

ii. a light chain complementarity-determining region 2 (CDR-L2) comprising or consisting of SEQ ID NO: 54 or a variant thereof, wherein in said variant up to 3 amino acids have been altered , for example wherein 2 or 1 amino acid(s) have been altered ; and

iii. a light chain complementarity-determining region 3 (CDR-L3) comprising or consisting of SEQ ID NO: 55 or a variant thereof, wherein in said variant up to 3 amino acids have been altered , for example wherein 2 or 1 amino acid(s) have been altered ;

or

a) a heavy chain variable (VH) region comprising or consisting of any of SEQ ID NO: 16, 8, 24, 32, 40 or 48 or a variant thereof, wherein in said variant up to 5 amino acids have been altered , for example wherein 4, 3, 2, or 1 amino acid(s) have been altered ; and

b) a light chain variable (VL) region comprising or consisting of any of SEQ ID NO: 20, 12, 28, 36, 44 and 52 or a variant thereof, wherein in said variant up to 5 amino acids have been altered , for example wherein 4, 3, 2, or 1 amino acid(s) have been altered ;

or

a) a heavy chain variable (VH) region comprising or consisting of SEQ ID NO: 16, or a variant thereof, wherein in said variant up to 5 amino acids have been altered , for example wherein 4, 3, 2, or 1 amino acid(s) have been altered ; and

b) a light chain variable (VL) region comprising or consisting of SEQ ID NO: 20, or a variant thereof, wherein in said variant up to 5 amino acids have been altered , for example wherein 4, 3, 2, or 1 amino acid(s) have been altered ;

or

a) a heavy chain variable (VH) region comprising or consisting of SEQ ID NO: 8, or a variant thereof, wherein in said variant up to 5 amino acids have been altered , for example wherein 4, 3, 2, or 1 amino acid(s) have been altered ; and

b) a light chain variable (VL) region comprising or consisting of SEQ ID NO: 12, or a variant thereof, wherein in said variant up to 5 amino acids have been altered , for example wherein 4, 3, 2, or 1 amino acid(s) have been altered ;

or

a) a heavy chain variable (VH) region comprising or consisting of SEQ ID NO: 24, or a variant thereof, wherein in said variant up to 5 amino acids have been altered , for example wherein 4, 3, 2, or 1 amino acid(s) have been altered ; and

b) a light chain variable (VL) region comprising or consisting of SEQ ID NO: 28, or a variant thereof, wherein in said variant up to 5 amino acids have been altered , for example wherein 4, 3, 2, or 1 amino acid(s) have been altered ;

or

a) a heavy chain variable (VH) region comprising or consisting of SEQ ID NO: 32, or a variant thereof, wherein in said variant up to 5 amino acids

have been altered , for example wherein 4, 3, 2, or 1 amino acid(s) have been altered ; and

b) a light chain variable (VL) region comprising or consisting of SEQ ID NO: 36, or a variant thereof, wherein in said variant up to 5 amino acids have been altered , for example wherein 4, 3, 2, or 1 amino acid(s) have been altered ;

or

a) a heavy chain variable (VH) region comprising or consisting of SEQ ID NO: 40, or a variant thereof, wherein in said variant up to 5 amino acids have been altered , for example wherein 4, 3, 2, or 1 amino acid(s) have been altered ; and

b) a light chain variable (VL) region comprising or consisting of SEQ ID NO: 44, or a variant thereof, wherein in said variant up to 5 amino acids have been altered , for example wherein 4, 3, 2, or 1 amino acid(s) have been altered ;

or

a) a heavy chain variable (VH) region comprising or consisting of SEQ ID NO: 48, or a variant thereof, wherein in said variant up to 5 amino acids have been altered , for example wherein 4, 3, 2, or 1 amino acid(s) have been altered ; and

b) a light chain variable (VL) region comprising or consisting of SEQ ID NO: 52, or a variant thereof, wherein in said variant up to 5 amino acids have been altered , for example wherein 4, 3, 2, or 1 amino acid(s) have been altered .

6. The antigen-binding protein according to any one of the preceding claims, wherein said antigen-binding protein comprises or consists of:

a) a heavy chain variable (VH) region comprising:

i. a heavy chain complementarity-determining region 1 (CDR-H1) comprising or consisting of SEQ ID NO: 17;

ii. a heavy chain complementarity-determining region 2 (CDR-H2) comprising or consisting of SEQ ID NO: 18; and

iii. a heavy chain complementarity-determining region 3 (CDR-H3) comprising or consisting of SEQ ID NO: 19; and

b) a light chain variable (VL) region comprising:

iv. a light chain complementarity-determining region 1 (CDR-L1) comprising or consisting of SEQ ID NO: 21;

v. a light chain complementarity-determining region 2 (CDR-L2) comprising or consisting of SEQ ID NO: 22; and

vi. a light chain complementarity-determining region 3 (CDR-L3) comprising or consisting of SEQ ID NO: 23;

or

a) a heavy chain variable (VH) region comprising:

i. a heavy chain complementarity-determining region 1 (CDR-H1) comprising or consisting of SEQ ID NO: 9;

ii. a heavy chain complementarity-determining region 2 (CDR-H2) comprising or consisting of SEQ ID NO: 10; and

iii. a heavy chain complementarity-determining region 3 (CDR-H3) comprising or consisting of SEQ ID NO: 11; and

b) a light chain variable (VL) region comprising:

iv. a light chain complementarity-determining region 1 (CDR-L1) comprising or consisting of SEQ ID NO: 13;

v. a light chain complementarity-determining region 2 (CDR-L2) comprising or consisting of SEQ ID NO: 14; and

vi. a light chain complementarity-determining region 3 (CDR-L3) comprising or consisting of SEQ ID NO: 15;

or

a) a heavy chain variable (VH) region comprising:

i. a heavy chain complementarity-determining region 1 (CDR-H1) comprising or consisting of SEQ ID NO: 33;

ii. a heavy chain complementarity-determining region 2 (CDR-H2) comprising or consisting of SEQ ID NO: 34; and

iii. a heavy chain complementarity-determining region 3 (CDR-H3) comprising or consisting of SEQ ID NO: 35; and

b) a light chain variable (VL) region comprising:

iv. a light chain complementarity-determining region 1 (CDR-L1) comprising or consisting of SEQ ID NO: 37;

v. a light chain complementarity-determining region 2 (CDR-L2) comprising or consisting of SEQ ID NO: 38; and

vi. a light chain complementarity-determining region 3 (CDR-L3) comprising or consisting of SEQ ID NO: 39.

7. The antigen-binding protein according to any one of the preceding claims, wherein said antigen-binding protein comprises or consists of:

a) a heavy chain variable (VH) region comprising or consisting of SEQ ID NO: 16; and

b) a light chain variable (VL) region comprising or consisting of SEQ ID NO: 20;

or

a) a heavy chain variable (VH) region comprising or consisting of SEQ ID NO: 8; and

b) a light chain variable (VL) region comprising or consisting of SEQ ID NO: 12;

or

a) a heavy chain variable (VH) region comprising or consisting of SEQ ID NO: 32; and

b) a light chain variable (VL) region comprising or consisting of SEQ ID NO: 36.

8. The antigen-binding protein according to any one of the preceding claims, wherein the antigen-binding protein is capable to bind to an epitope contained

between residues corresponding to amino acids 24 to 60 of the toxin protein sequences as defined in any one of SEQ:IDs No 56, 57, 58, 59 and 60 or variant(s) thereof having at least 70% identity, such as at least 75% identity, for example at least 80% identity, such as at least 85% identity, for example at least 90% identity, such as at least 95% identity thereto, preferably wherein the antigen-binding protein is capable to bind to an epitope contained between residues corresponding to amino acids 41 to 60 of the toxin protein sequences as defined in any one of SEQ:IDs No 56, 57, 58, 59 and 60, or SEQ:IDs No 65, 66, 66, 67 and 68 respectively, or variant(s) thereof having at least 70% identity, such as at least 75% identity, for example at least 80% identity, such as at least 85% identity, for example at least 90% identity, such as at least 95% identity thereto.

9. The antigen-binding protein according to any one of the preceding items, wherein the antigen-binding protein is capable to bind to an epitope comprising or consisting of the sequence: CAATCPXVKXGVXIXCCST (SEQ ID NO: 69), wherein X may be any amino acid.

10. A pharmaceutical composition comprising an antigen-binding protein as defined in any one of the preceding claims, and a pharmaceutically acceptable diluent, carrier and/or excipient, optionally further comprising one or more additional antigen-binding protein(s) capable of binding to, blocking, or neutralizing one or more snake venom antigen(s).

11. A kit-of-parts comprising
    a) an antigen-binding protein according to anyone of claims 1 to 9; and
    b) another agent suitable for the treatment of snake envenomation, preferably wherein said other agent used for the treatment of snake envenomation is one or more additional antigen-binding protein(s) selected from the group consisting of: IgG, IgM, scFv, $F(ab)_2$, Fab, V_HH antigen-binding protein(s), and fragments thereof, even more preferably wherein the one or more additional antigen-binding protein(s) capable of neutralizing one or more snake venom antigen(s) is (are) selected from the group consisting of: Equine immunoglobulin(s) against snake venom, such as Equine IgG(s) or Equine Fab or Equine $F(ab')_2$

fragment(s) against snake venom, Ovine immunoglobulin(s) against snake venom, such as Ovine IgG(s) or Ovine Fab or Ovine $F(ab')_2$ fragment(s) against snake venom, Rabbit immunoglobulin(s) against snake venom, such as Rabbit IgG(s) or Rabbit Fab or Rabbits $F(ab')_2$ fragment(s) against snake venom, and Camelids immunoglobulin(s) against snake venom, such as Camelids IgG(s) or Camelids Fab or Camelids $F(ab')_2$ fragment(s) against snake venom.

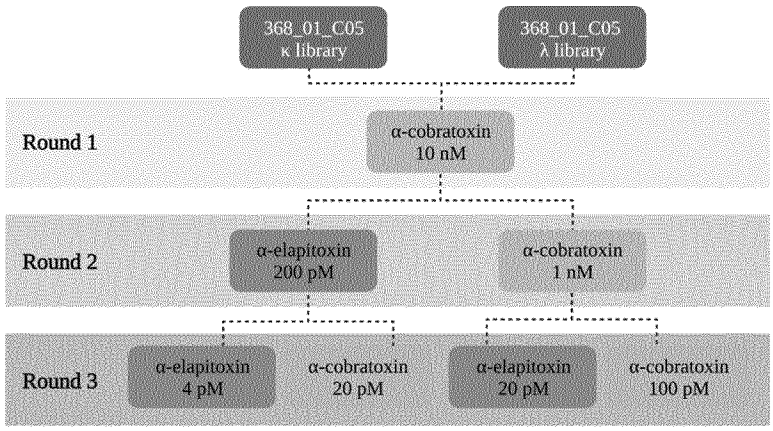
12. An antigen-binding protein according to any one of claims 1 to 9, a pharmaceutical composition according to claim 10, or a kit-of-parts according to claim 11, for use in a method of treatment of snake envenomation in a subject.

13. A kit for detection and/or quantification of snake venom in a sample comprising:
- one or more antigen-binding proteins, such as two antigen-binding proteins, wherein at least one of said antigen-binding proteins is according to any one of claims 1 to 9; and
- means for detection of a complex comprising said one or more antigen-binding proteins bound to snake venom.

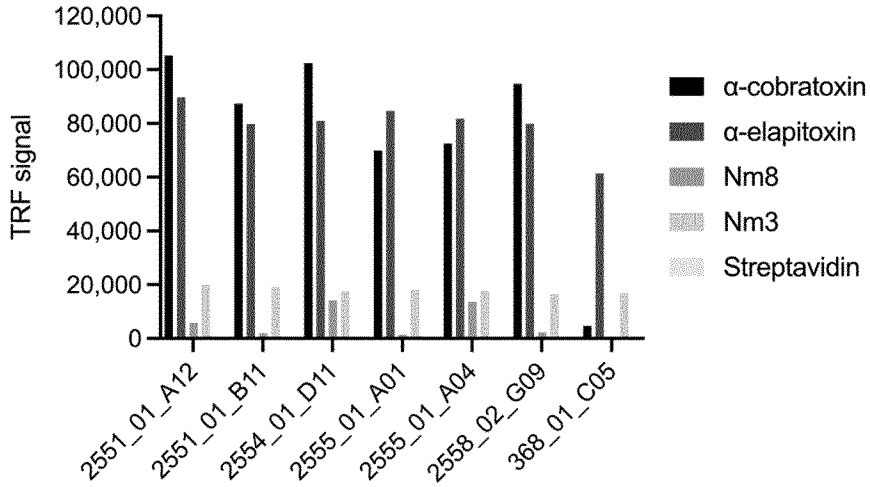
14. Use of an antigen-binding protein according to any one of claims 1 to 9 in an *in vitro* method for detection and/or diagnosis of snake envenomation in a subject.

15. A method for detecting snake venom in a sample, said method comprising:
- providing a sample for analysis;
- contacting the sample with one or more antigen-binding proteins as defined in any one of claims 1 to 9 to said sample; and
- detecting a complex comprising said one or more antigen-binding proteins bound to a snake antigen.

A.



B.



C.

Antibody ID	Gene	CDR-L1	CDR-L2	CDR-L3
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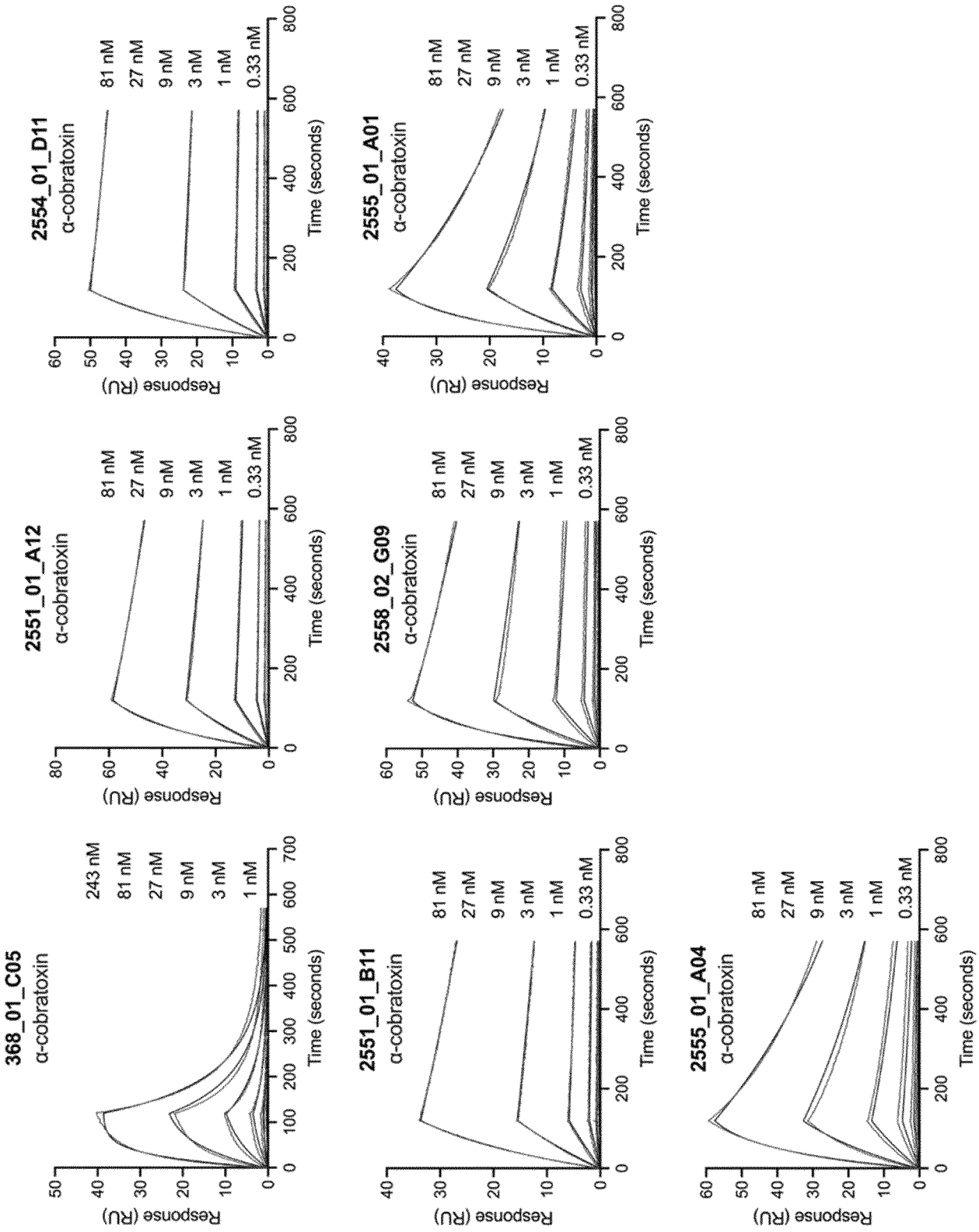
2551_01_A12	IGLV6-57	SGRIVSDY	EDN	QSYDSSNAYVV
2554_01_D11	IGLV6-57	SGSIGSDY	EDN	QSYDRSNHEVV
2551_01_B11	IGLV3-21	--NIGSNI	HNT	QVWDSSSEHVV
2555_01_A01	IGLV3-21	--NIGQQI	SDS	QVWDSGSDHVV
2555_01_A04	IGLV3-21	--YIGGES	DDT	QVWDVSSDHVV
2558_02_G09	IGLV3-21	--NIGQQI	DGS	QVWDITSDHVV

Fig. 1

A.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27 nM
9 nM
3 nM
1 nM
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α-cobratoxin
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27 nM
9 nM
3 nM
1 nM
0.33 nM
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α-cobratoxin
81 nM
27 nM
9 nM
3 nM
1 nM
0.33 nM
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α-cobratoxin
81 nM
27 nM
9 nM
3 nM
1 nM
0.33 nM
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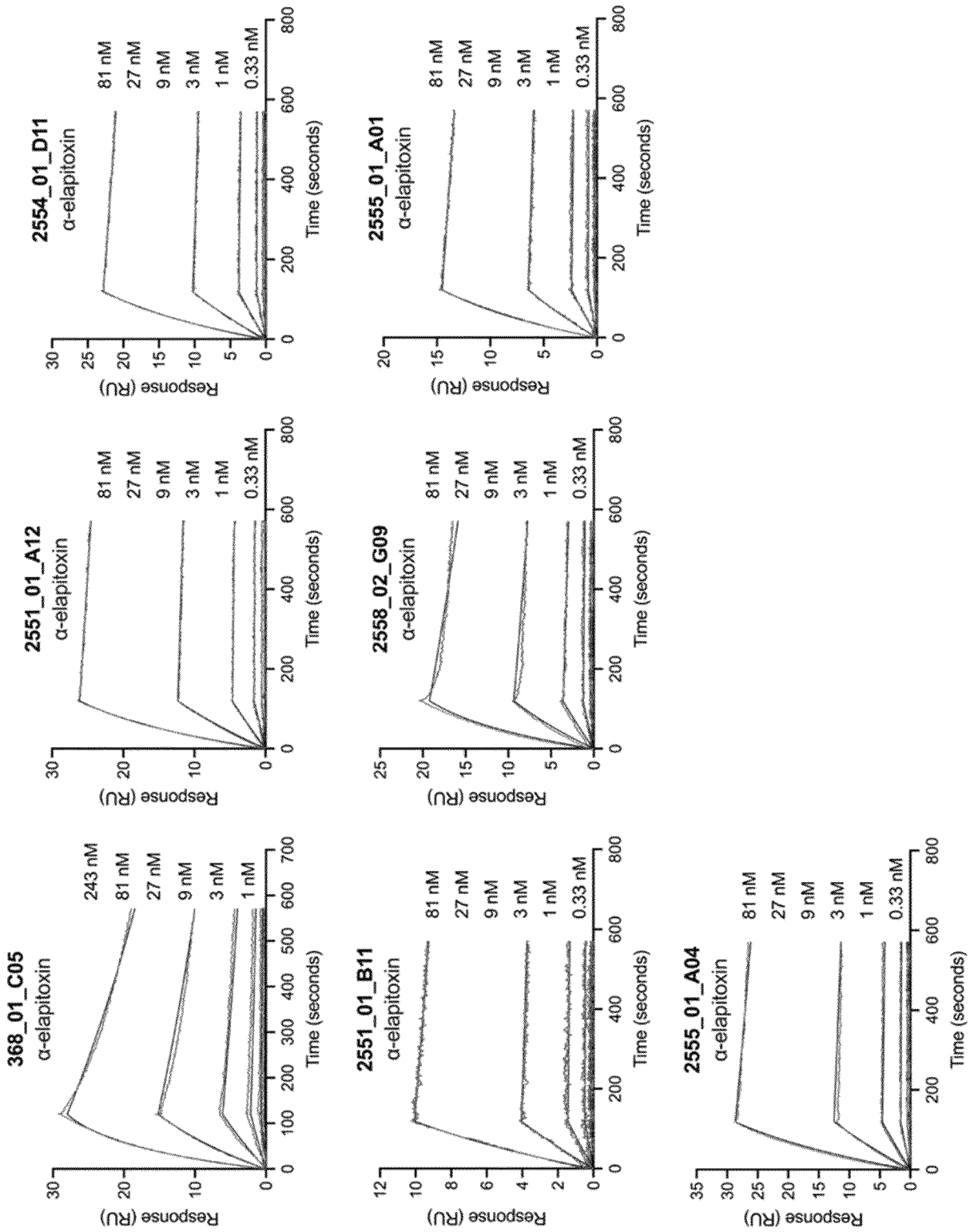
α-cobratoxin
81 nM
27 nM
9 nM
3 nM
1 nM
0.33 nM
2555_01_A01
α-cobratoxin
81 nM
27 nM
9 nM
3 nM
1 nM
0.33 nM
2555_01_A04
α-cobratoxin
81 nM
27 nM
9 nM
3 nM
1 nM
0.33 nM
Response (RU)
Time (seconds)

Fig. 2

B.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α-elapitoxin
243 nM
81 nM
27 nM
9 nM
3 nM
1 nM
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27 nM
9 nM
3 nM
1 nM
0.33 nM
2554_01_D11
α-elapitoxin
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27 nM
9 nM
3 nM
1 nM
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α-elapitoxin
81 nM
27 nM
9 nM
3 nM
1 nM
0.33 nM
2558_02_G09
α-elapitoxin
81 nM
27 nM
9 nM
3 nM
1 nM
0.33 nM
2551_01_B11
α-elapitoxin
81 nM
27 nM
9 nM
3 nM
1 nM
0.33 nM
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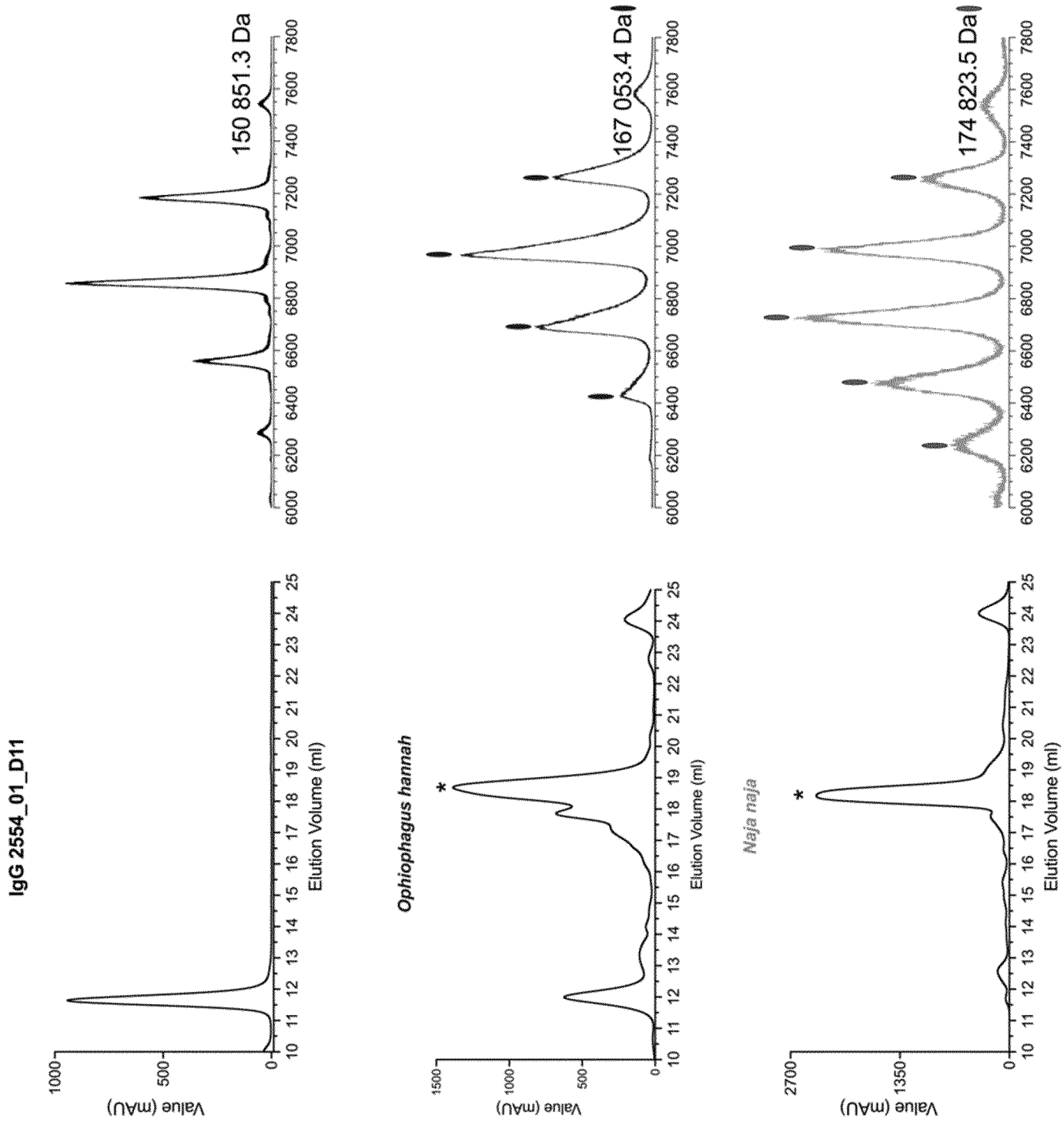
9 nM
3 nM
1 nM
0.33 nM
Response (RU)
Time (seconds)

Fig. 2, cont.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Ophiophagus hannah
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Naja naja
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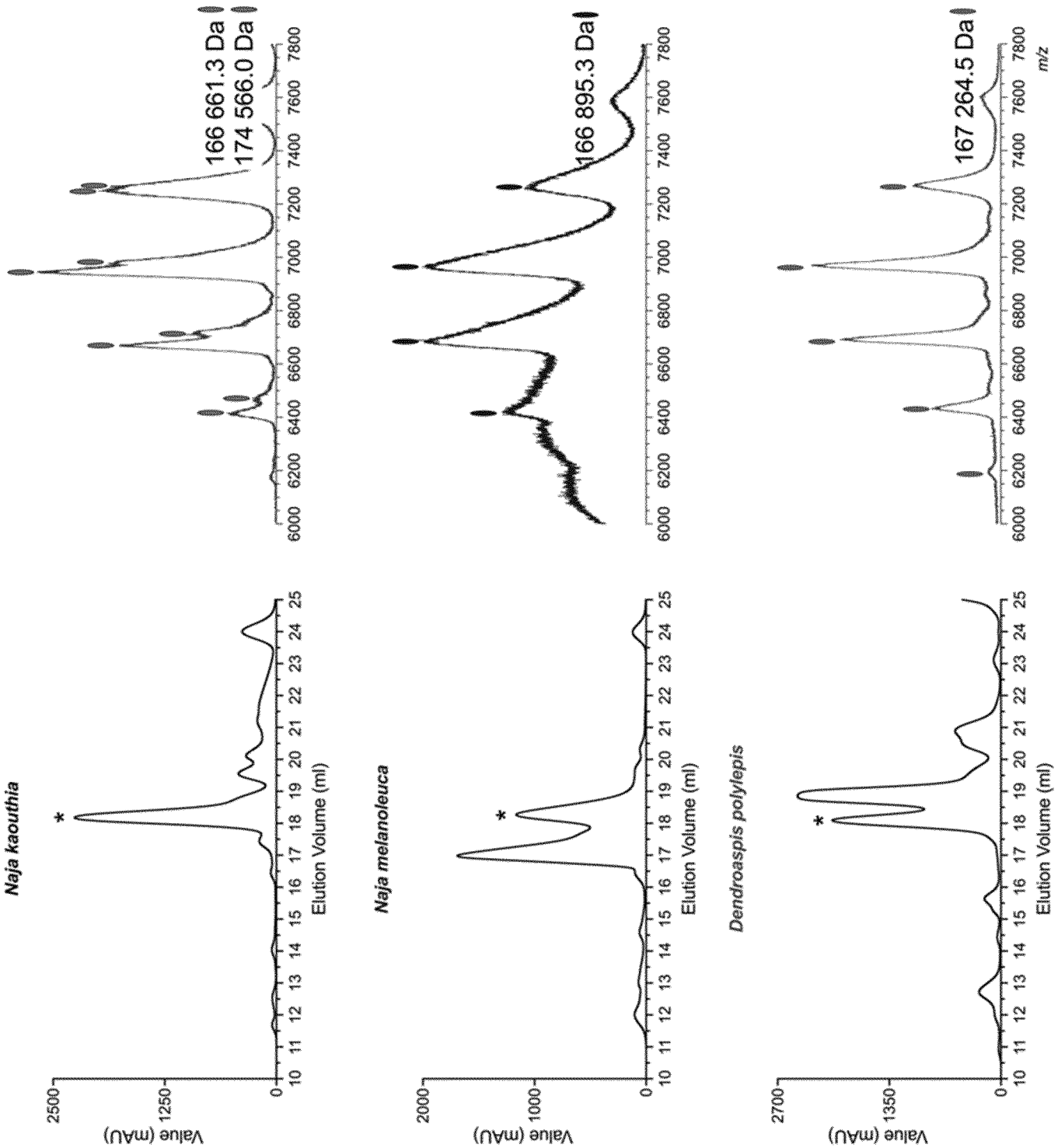
Value (mAU)
Elution Volume (ml)
*

Fig. 3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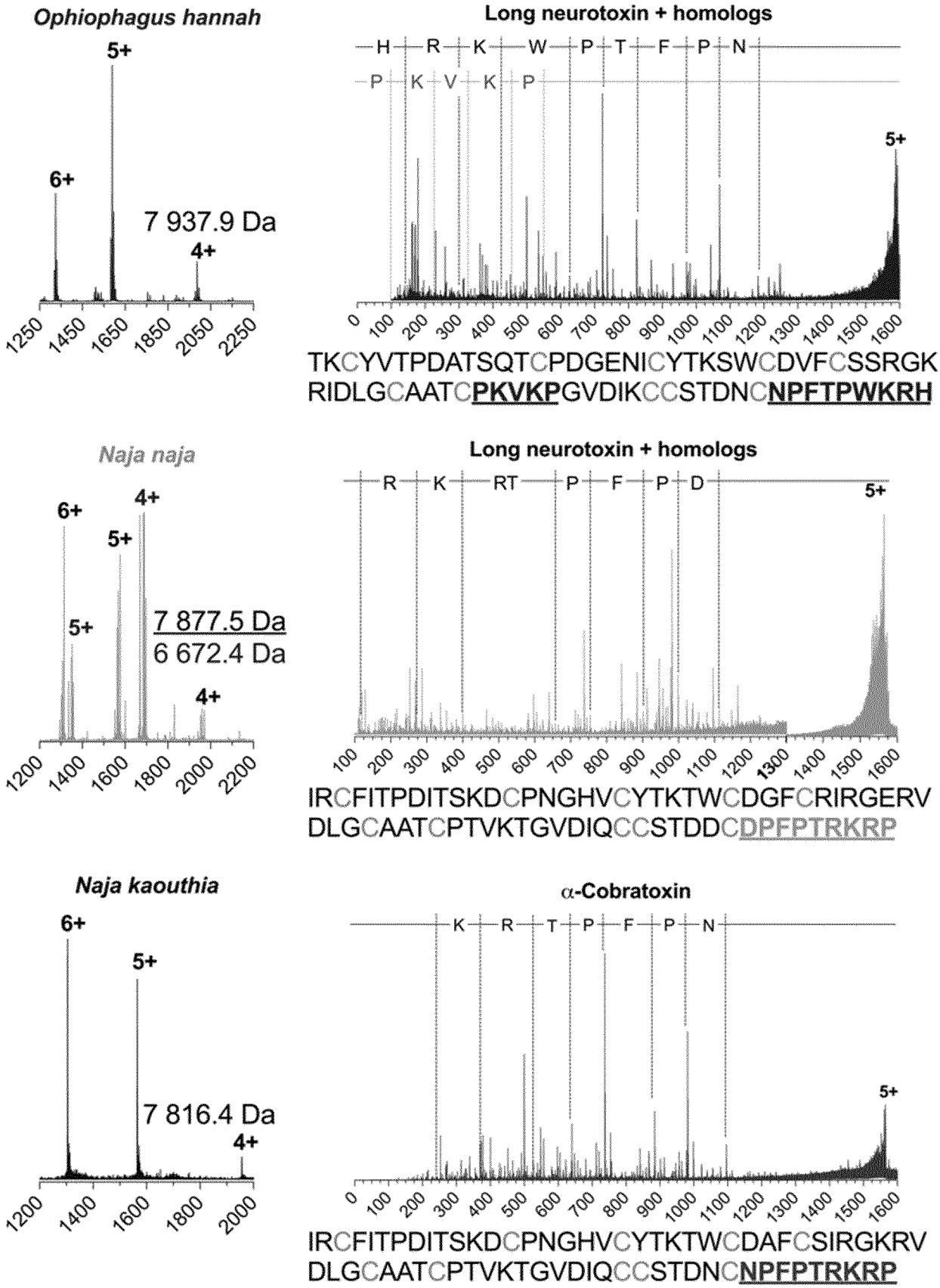
Value (mAU)
Elution Volume (ml)
m/z
*

Fig. 3, cont.


Ophiophagus hannah
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6+
7 937.9 Da
4+
1250 1450 1650 1850 2050 2250
Long neurotoxin + homologs
H R K W P T F P N
P K V K P
5+
0 100 200 300 400 500 600 700 800 900 1000 1100 1200 1300 1400 1500 1600
TKCYVTPDATSQTCPDGENICYTKSWCDVFCSSRGK
RIDLGCAATCPKVKPGVDIKCCSTDNCNPFTPWKRH
Naja naja
6+
4+
5+
5+
7 877.5 Da
6 672.4 Da
4+
1200 1400 1600 1800 2000 2200
Long neurotoxin + homologs
R K RT P F P D
5+
100 200 300 400 500 600 700 800 900 1000 1100 1200 1300 1400 1500 1600
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5+
0 100 200 300 400 500 600 700 800 900 1000 1100 1200 1300 1400 1500 1600
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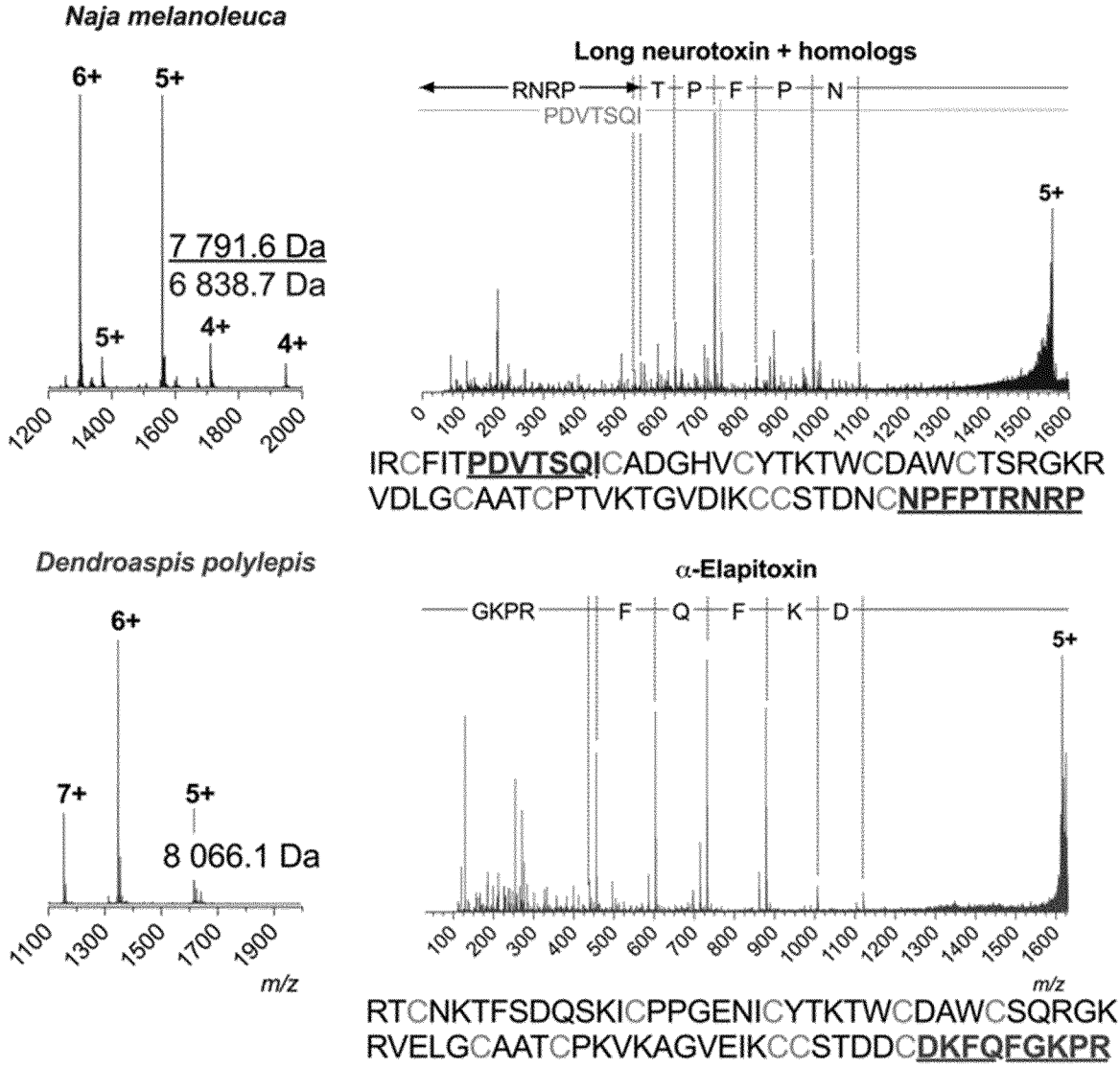
Fig. 4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5+
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6 838.7 Da
5+
4+
4+
1200
1400
1600
1800
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Long neurotoxin + homologs
RNRP
T
P
F
P
N
PDVTSQI
5+
0
100
200
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7+
5+
8 066.1 Da
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Q
F
K
D
5+
100
200
300
400
500
600
700
800
900
1000
1100
1200
1300
1400
1500
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m/z
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Fig. 4, cont.

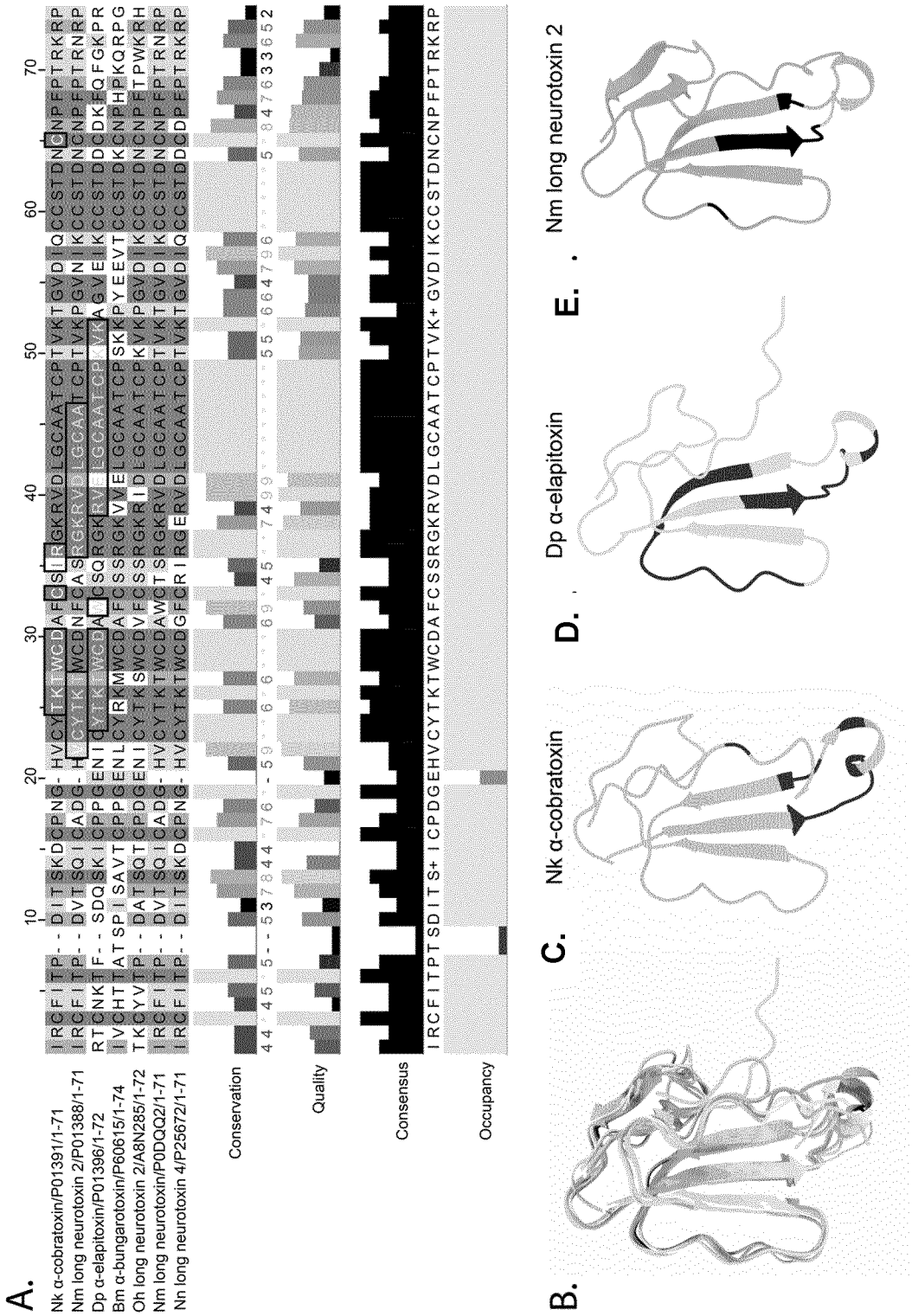
A.
Nk α-cobratoxin/P01391/1-71
Nm long neurotoxin 2/P01388/1-71
Dp α-elapitoxin/P01396/1-72
Bm α-bungarotoxin/P60615/1-74
Oh long neurotoxin 2/A8N285/1-72
Nm long neurotoxin/P0DQQ2/1-71
Nn long neurotoxin 4/P25672/1-71
Conservation
Quality
Consensus
Occupancy
B.
C. Nk α-cobratoxin
D. Dp α-elapitoxin
E. Nm long neurotoxin 2

Fig. 5

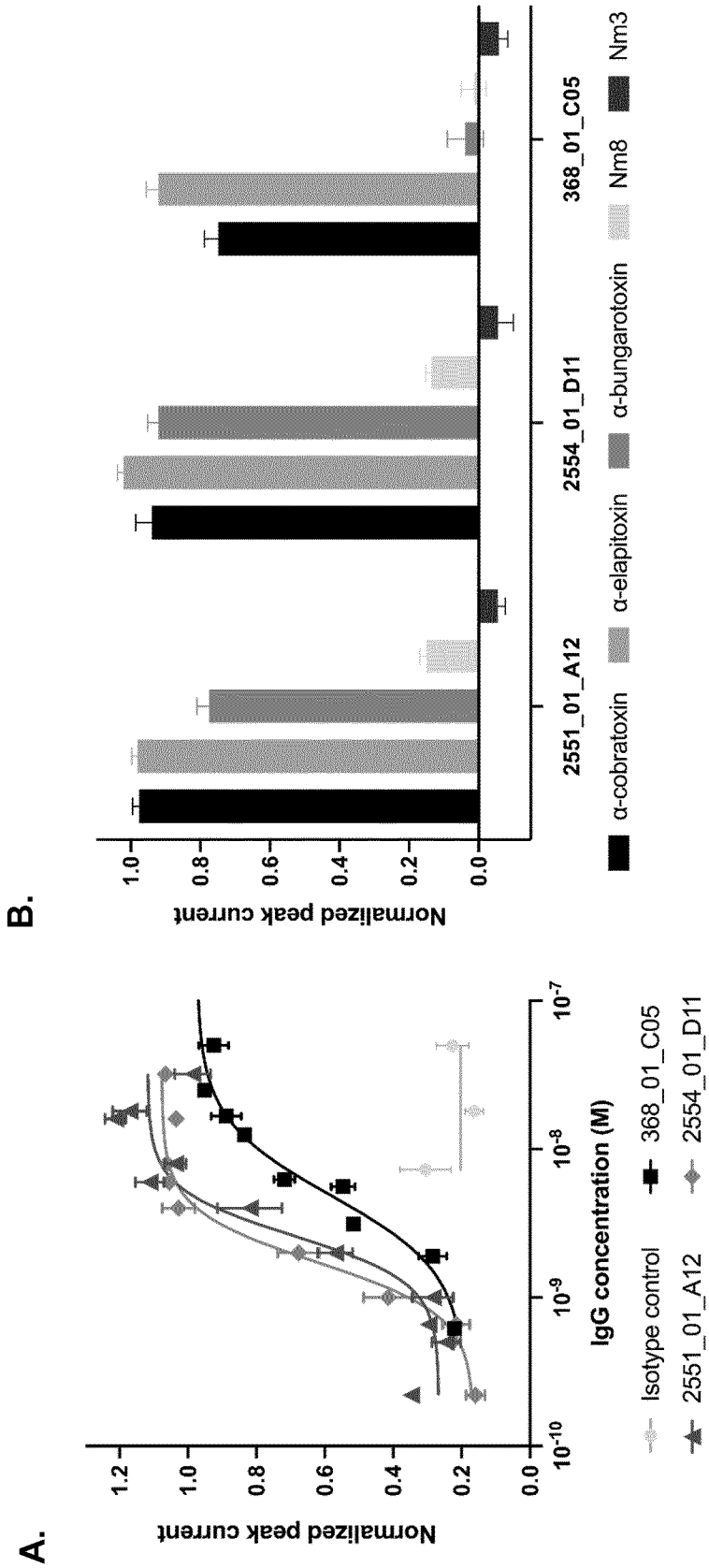
B.
Normalized peak current
1.0
0.8
0.6
0.4
0.2
0.0
2551_01_A12
2554_01_D11
368_01_C05
α-cobratoxin
α-elapitoxin
α-bungarotoxin
Nm8
Nm3
A.
Normalized peak current
1.2
1.0
0.8
0.6
0.4
0.2
0.0
10-10
10-9
10-8
10-7
IgG concentration (M)
Isotype control
368_01_C05
2551_01_A12
2554_01_D11

Fig. 6

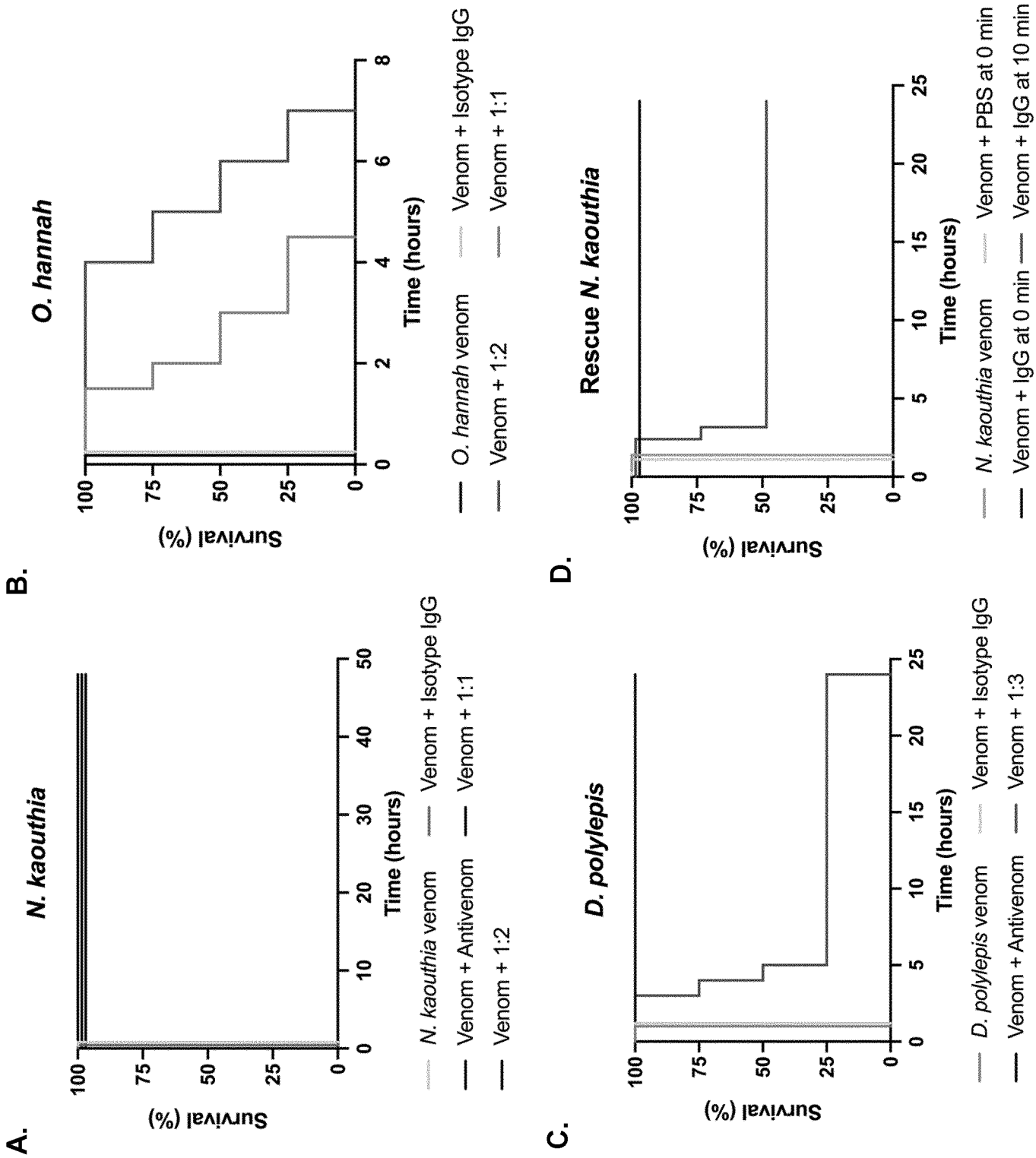
A.
N. kaouthia
Survival (%)
Time (hours)
N. kaouthia venom
Venom + Antivenom
Venom + 1:2
Venom + Isotype IgG
Venom + 1:1
B.
O. hannah
Survival (%)
Time (hours)
O. hannah venom
Venom + 1:2
Venom + Isotype IgG
Venom + 1:1
C.
D. polylepis
Survival (%)
Time (hours)
D. polylepis venom
Venom + Antivenom
Venom + Isotype IgG
Venom + 1:3
D.
Rescue N. kaouthia
Survival (%)
Time (hours)
N. kaouthia venom
Venom + IgG at 0 min
Venom + PBS at 0 min
Venom + IgG at 10 min

Fig. 7

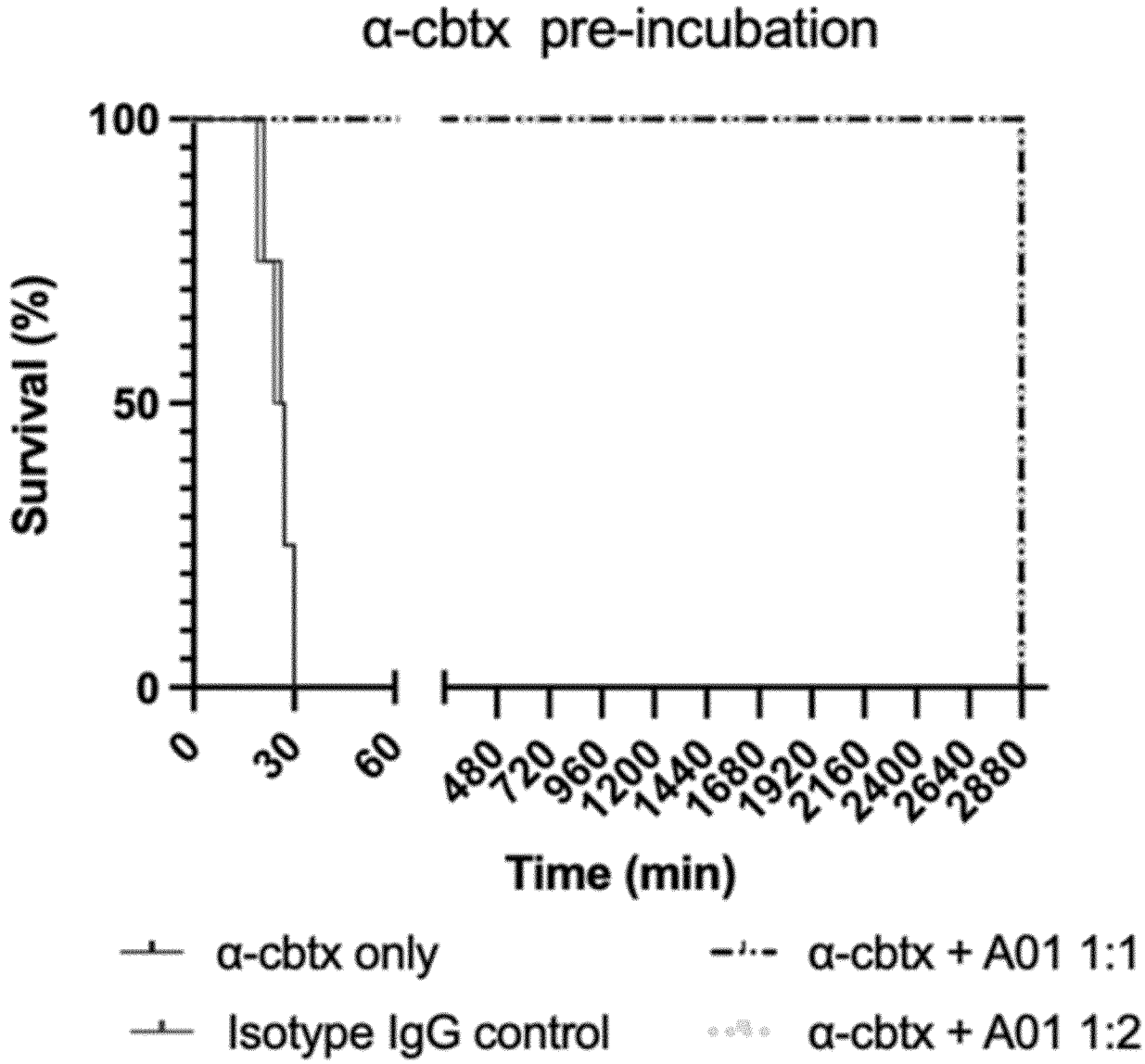
α-cbtx pre-incubation
Survival (%)
100
50
0
0
30
60
480
720
960
1200
1440
1680
1920
2160
2400
2640
2880
Time (min)
α-cbtx only
α-cbtx + A01 1:1
Isotype IgG control
α-cbtx + A01 1:2

Fig. 8

INTERNATIONAL SEARCH REPORT

International application No.

PCT/EP2023/061365

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. [X] forming part of the international application as filed.

   b. [ ] furnished subsequent to the international filing date for the purposes of international search (Rule 13*ter*.1(a)).

      [ ] accompanied by a statement to the effect that the sequence listing does not go beyond the disclosure in the international application as filed.

2. [ ] With regard to any nucleotide and/or amino acid sequence disclosed in the international application, this report has been established to the extent that a meaningful search could be carried out without a WIPO Standard ST.26 compliant sequence listing.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2023/061365

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K16/18 A61P39/02
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61P A61K C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Ledsgaard Jensen Line: "Discovery of a broadly-neutralizing human antibody that can rescue mice challenged with neurotoxin-rich snake venoms", , 1 February 2022 (2022-02-01), XP93069456, Retrieved from the Internet: URL:https://backend.orbit.dtu.dk/ws/portalfiles/portal/310617703/20220228_PhD_thesis_Line_Ledsgaard.pdf page 126 - page 156 ----- -/--	1-15

[x] Further documents are listed in the continuation of Box C.

[x] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

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"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance;; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance;; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
3 August 2023	11/08/2023

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Mattugini, Nicola

2

Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Ledsgaard Line ET AL: "In vitro discovery and optimization of a human monoclonal antibody that neutralizes neurotoxicity and lethality of cobra snake venom", bioRxiv, 7 September 2021 (2021-09-07), XP093012397, DOI: 10.1101/2021.09.07.459075 Retrieved from the Internet: URL:https://www.biorxiv.org/content/10.1101/2021.09.07.459075v1 [retrieved on 2023-01-09] abstract; page 6 - page 7; figure 1	1-15
Y	AHMADI SHIRIN ET AL: "An in vitro methodology for discovering broadly-neutralizing monoclonal antibodies", SCIENTIFIC REPORTS , vol. 10, no. 1 1 July 2020 (2020-07-01), XP055967569, DOI: 10.1038/s41598-020-67654-7 Retrieved from the Internet: URL:http://www.nature.com/articles/s41598-020-67654-7 page 1 - page 7	1-15
A	TREMEAU O ET AL: "A monoclonal antibody which recognized the functional site of snake neurotoxins and which neutralizes all short-chain variants", FEBS LETTERS, ELSEVIER, AMSTERDAM, NL, vol. 208, no. 2, 24 November 1986 (1986-11-24), pages 236-240, XP025603921, ISSN: 0014-5793, DOI: 10.1016/0014-5793(86)81024-9 [retrieved on 1986-11-24] page 1 - page 5	1-15
A	KINI R M ET AL: "Structure, function and evolution of three-finger toxins: Mini proteins with multiple targets", TOXICON, ELMSFORD, NY, US, vol. 56, no. 6, 1 November 2010 (2010-11-01), pages 855-867, XP027242522, ISSN: 0041-0101 [retrieved on 2010-07-27] the whole document	1-15

-/--

2

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	Ledsgaard Line ET AL: "Discovery of a broadly-neutralizing human antibody that can rescue mice challenged with neurotoxin-rich snake venoms", bioRxiv, 17 June 2022 (2022-06-17), XP093012403, DOI: 10.1101/2022.06.17.496531 Retrieved from the Internet: URL:https://www.biorxiv.org/content/10.1101/2022.06.17.496531v1 [retrieved on 2023-01-09] the whole document -----	1-15
X,P	WO 2022/217116 A1 (CENTIVAX INC [US]) 13 October 2022 (2022-10-13) the whole document -----	1-15

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2023/061365

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2022217116 A1	13-10-2022	NONE	