Guiding recombinant antivenom development by omics technologies

Laustsen, Andreas Hougaard

Published in:
New Biotechnology

Link to article, DOI:
10.1016/j.nbt.2017.05.005

Publication date:
2017

Document Version
Peer reviewed version

Manuscript Details

Manuscript number	NBT_2017_136
Title	Guiding recombinant antivenom development by omics technologies
Article type	Review Article

Abstract

In this review, the different approaches that have been employed with the aim of developing novel antivenoms against animal envenomings are presented and discussed. Reported efforts have focused on the use of innovative immunization strategies, small molecule inhibitors against enzymatic toxins, endogenous animal proteins with toxin-neutralizin capabilities, and recombinant monoclonal antibodies. Harnessing either of these approaches, antivenom development may benefit from an in-depth understanding of venom compositions and which toxins that are essential to neutralize in an envenoming case. Focus is thus also directed towards the different omics technologies (particularly venomics, antivenomics, and toxicovenomics) that are being used to uncover novel animal toxins, shed light on venom complexity, and provide directions for how to determine the medical relevance of individual toxins within whole venoms. Finally, techniques for assessing antivenom specificity and cross-reactivity are reviewed, with special focus on antivenomics and high-density peptide microarray technology.

Keywords	Toxinology; recombinant antivenom; toxicovenomics; antivenomics; high-density peptide microarray technology;
Corresponding Author	Andreas Laustsen
Corresponding Author's Institution	Technical University of Denmark
Order of Authors	Andreas Laustsen
Suggested reviewers	Juan J. Calvete, Manuela Pucca, Nicholas Casewell, Bruno Lomonte

Submission Files Included in this PDF

File Name [File Type]

Cover letter, New Biotechnology.docx [Cover Letter]

Laustsen. Antibody discovery guided by venom proteomics v5.doc [Manuscript File]

Highlights.docx [Highlights]

Submission Files Not Included in this PDF

File Name [File Type]

Figure 1. Toxicovenomics.png [Figure]

Figure 2. Antivenomics.png [Figure]

Figure 3, peptide microarrays.png [Figure]

To view all the submission files, including those not included in the PDF, click on the manuscript title on your EVISE Homepage, then click 'Download zip file'.

April 12th, 2017
Dr. Mike Taussig
Editor-in-Chief
New Biotechnology

Dear Dr. Mike Taussig

Thank you for your encouragement to publish in the journal. Please find attached the manuscript entitled '*Guiding recombinant antivenom development by omics technologies'*, to be considered for publication as a review article in New Biotechnology.

In recent year, there has been an increased focus on how to overcome the challenge of snakebite and bring novel antivenoms to poor rural parts of the tropical world. A few reviews are already available in the field (of which I have co-authored some), however, these are focused on the individual efforts and specific toxin-neutralizing molecules, antibodies, or sera, and fail to provide an overview of the omics technologies employed to guide their development. Here, I have aimed to provide such an overview, and bring more clarity to how omics technologies interplay with toxinology and how these may be employed to guide medicinal chemistry and biotechnological efforts within antivenom development. It is my personal experience that too many research efforts have been unsuccessful, as they have attempted to develop toxin-targeting molecules without taking the complexity of venom and the often inverse relationship between toxicity and immunogenicity into account. It is my belief that an overview of how omics technologies can be harnessed in antivenom development will be useful for particularly drug development researchers (and graduate students) who are new in the field, and who need to gain an understanding of venoms and how these may be viewed and analyzed as drug targets.

I therefore hope that you will take this article into consideration.

Sincerely yours,

Dr. Andreas H. Laustsen

Guiding recombinant antivenom development by omics technologies

Andreas H. Laustsen

Department of Biotechnology and Biomedicine, Technical University of Denmark, Kongens Lyngby, Denmark

Address for correspondence:

Dr. Andreas H. Laustsen

Postdoctoral Fellow

Department of Biotechnology and Biomedicine

Technical University of Denmark

DK-2800 Kgs. Lyngby, Denmark

*ahola@bio.dtu.dk

Abstract

In this review, the different approaches that have been employed with the aim of developing novel antivenoms against animal envenomings are presented and discussed. Reported efforts have focused on the use of innovative immunization strategies, small molecule inhibitors against enzymatic toxins, endogenous animal proteins with toxin-neutralizin capabilities, and recombinant monoclonal antibodies. Harnessing either of these approaches, antivenom development may benefit from an in-depth understanding of venom compositions and which toxins that are essential to neutralize in an envenoming case. Focus is thus also directed towards the different omics technologies (particularly venomics, antivenomics, and toxicovenomics) that are being used to uncover novel animal toxins, shed light on venom complexity, and provide directions for how to determine the medical relevance of individual toxins within whole venoms. Finally, techniques for assessing antivenom specificity and cross-reactivity are reviewed, with special focus on antivenomics and high-density peptide microarray technology.

Introduction

Among the tropical diseases, snakebite envenoming remains one of the most neglected, causing mortality and morbidity to thousands of victims worldwide each year [1–3]. In addition, envenomings by other species, particularly scorpions and spiders, also constitute a medically important challenge for public health [4–7]. Modern approaches based on biotechnology and medicinal chemistry are starting to see the light of day through neutralization of animal toxins by monoclonal antibodies (mAbs) and small molecule inhibitors [8,9]. Yet, serum-based antivenom derived from immunized animals is currently the only therapeutically effective treatment option against most animal envenomings [10]. Antivenom is thus one of the few biological therapies that have not yet entered the modern era of biologics, despite the presence of an overwhelmingly large patient population. Although part of the explanation for the lack of innovation within antivenom development may be attributed to the poor financial incentive for investment in the field, the sheer complexity of animal venoms may also hold part of the answer. Not only is each venom a complex mixture of toxins, but venoms are highly diverse across the known 725 venomous snake species [8], 2000 scorpion species, and 44,000 spider species [9]. As an example and rough estimation, it has previously been suggested that between 19,000-25,000 toxins may exist in the venoms of the two snake families, Elapidae and Viperidae, that contain the species of the highest medical relevance for human health [8]. Although neutralization of many of these toxins and toxins from other animal species may not individually be medically essential in every envenoming case [11], such numbers strongly support the notion that animal venoms are among the most complex drug targets known to man. In this review, novel strategies for developing modern envenoming therapies are reviewed with special focus on how omics (particularly venomics [12] and toxicovenomics [13]) technologies can be employed to guide discovery of antibodies capable of neutralizing medically relevant toxins.

Neutralization of animal toxins

Animal venom toxins are proteinaceous and have evolved primarily to subdue prey, as well as to deter predators. Animal toxins can exert a myriad of different pathophysiological effects in victims of envenoming, including systemic neurotoxicity, haemotoxicity, myotoxicity, and cytotoxicity, manifesting clinically as flaccid paralysis, involuntary muscle contraction, various coagulopathies, nephrotoxicity, and local tissue damage including necrosis [14,15]. Different approaches have been pursued in the attempt to combat different animal venoms and toxins, including the use of novel immunization methods, small molecule inhibitors, endogenous toxin-neutralizing animal proteins, and antibodies. These will be presented in the following. It is, however, beyond the scope of this article to provide an exhaustive review on all the examples of individual antitoxins and novel antisera that have been reported to date, as these can be found elsewhere [2,6,8,9].

Optimizing antisera by next generation immunization technology

Two of the challenges in current antivenom production include procurement of venoms and obtaining a balanced response against the medically relevant toxins within a venom [16]. Additionally, intraspecies venom variation is a common phenomenon [17,18], which may further complicate design of effective immunization mixtures. Although differences in antibody responses also occur among immunized animals for antivenoms, variation in response can to some extent be controlled using standardized immunogens, such as (multi)epitope DNA strings, synthetic peptides, or recombinant toxins [6]. Using such molecules also has the advantage that the immunization mixture can be designed to contain only those immunogens of medical relevance to humans. A prominent example of the successful use of DNA strings for immunization was reported in 2006, where antisera against toxins of the two vipers, *Echis ocellatus* and *Cerastes cerastes*, were successfully raised by immunizing mice in the epidermal layer of their abdominal region with a

multiepitope DNA immunogen using a GeneGun [19]. Using a synthetic peptide containing continuous and discontinuous epitopes derived from the *Centruroides noxius* (scorpion) Cn2 toxin, researchers in another study succeeded in raising efficacious antiserum in rabbits [20]. Similarly, rabbits have also been immunized with a recombinant, non-toxic version (a toxoid) of the TsNTxP toxin from *Tityus serrulatus* venom, which could protect rabbits against 20 LD_{50}s of whole venom from this scorpion [21]. Nevertheless, despite these and many more examples of the successful use of such modern immunization technologies, the final products of their use are still serum-based medicines that are costly to produce and may be immunogenic to human recipients due to their heterologous nature.

Using small molecule inhibitors against animal toxins

A few dozen molecules have been reported to show inhibitory effects against various toxins from spiders, scorpions, and snakes [8,9]. Common to all these small molecule inhibitors is that they target enzymatically active toxins, either by mimicking the natural substrate or scavenging an important co-factor for the apoenzyme. Examples of toxin-inhibiting small molecules include varespladib, which effectively neutralizes phospholipase A_2 activity of many snake venoms [22], batimastat and EDTA, which chelate Zn^{2+} ions and thereby inhibit metalloprotease activity [23], and heparin, which may inhibit hyaluronidase activity [24–26]. However, no toxin-targeting small molecule is currently in clinical use. More details and examples of small molecule inhibitors (particularly from plants) is beyond the scope of this review and can be found elsewhere [27].

Harnessing natural toxin-neutralizing proteins from animals

Non-antibody proteinaceous molecules capable of neutralizing animal toxins have not so far been reported outside the field of snake venoms. However, for snake venoms the presence of protective

proteins has been described in a range of different animal species, including the South American opossum, *Didelphis marsupialis* [28–30], snakes themselves [31–35], and ground squirrels (*Spermophilus beecheyi*) [36]. Some of these factors have even shown greater toxin-neutralizing capacity than commercial antivenoms, exemplified by the 97 kDa protein and the antibothropic complex derived from *Didelphis marsupialis* serum [30]. These proteins were 4 and 6 times more potent than the commercially available antivenom against *Bothrops lanceolatus* venom and *B. jararaca* venom, respectively [30]. Although such results are fascinating and may even seem promising, two major obstacles are likely to prevent the use of these types of molecules from being used as envenoming therapy. Firstly, the expression of non-standard protein formats is not always a trivial matter. Given the molecular diversity of the different naturally occurring toxin-neutralizing proteins, eventual expression/production would be resource-consuming to standardize (though not necessarily in the distant future). Secondly, none of the toxin-neutralizing proteins are of human origin; combined with the fact that many of the toxins are quite large (> 50 kDa) proteins, their heterologous nature is likely to inflict adverse reactions in human recipients due to the likelihood of high immunogenicity. Possibly, however, if a promising scaffold protein were to be developed with low immunogenicity and able to be engineered easily to target a multitude of different toxin families, it is conceivable that a molecular platform and discovery strategy could be employed for development of non-antibody-based toxin-neutralizing proteins. Examples of such platform technologies might include DARPins [37,38], Armadillo repeat proteins [39], affitins [40–42], adhirons [43], anticalins [44], and various other protein scaffolds [45], although these molecular formats are yet to be tested for their applicability for antitoxin development.

Employing monoclonal antibodies to neutralize animal toxins

Various different monoclonal antibody (mAb) formats have been discovered and developed against toxins from different animal species, including snakes, scorpions, spiders, and bees [8,9,46]. The first use of a mAb capable of neutralizing a toxin was reported in 1982 using hybridoma technology [47]. This IgG antibody targets toxin α from the Black-necked spitting cobra, *Naja nigricollis*. Since then, 63 additional murine IgG mAbs targeting toxins from snakes, spiders, and scorpions have been reported [8,9]. However, this number is unlikely to increase significantly, given the prospects of using transgenic (humanized) animals capable of producing human IgGs more suitable for human therapy and the advent of phage display technology. This is one of the most promising avenues for development of novel recombinant antivenoms [48,49]. Most commonly, phage display selection has been used to develop human single-chain variable fragments (scFvs) with important examples including the development of Serrumab against the toxins, Ts1 and Ts2, from the Brazilian yellow scorpion (*T. serrulatus*) [50,51], Afribumab targeting melittin and phospholipase A_2s in Africanized bee (*Apis mellifera*) venom [46], the human scFv P2B7 capable of neutralizing myotoxicity from *Bothrops jararacussu* venom [52], and ER-5 against β-neurotoxins from *Centruroides* scorpion venoms [53]. Other formats, including diabodies [54,55], camelid V_HH [56–60], bispecific V_HH [61], and V_HH-Fc fusions [62] have also been reported to successfully neutralize a range of different toxins, particularly from scorpions and snakes. The prospect of using (mixtures of) human mAbs against animal envenomings has gained increasing interest in recent years. The reasons for this include the versatility of the (human) antibody scaffold, its compatibility with the human immune system, the success of human antibodies in other fields [63], and the demonstration that recombinant antivenoms may be produced cost-competitively by mammalian cell cultivation [64,65] with future production costs of antibodies likely to decrease even further [66]. Despite the existence of several feasible discovery approaches (particularly using humanized

153 transgenic animals, phage display, or even combined approaches), a need remains to elucidate
154 which animal toxins to focus antivenom research efforts on.
155
156 **Using omics technologies to establish order in venom complexity**
157 Independent of the molecular scaffold and discovery strategy employed for developing the next
158 generation of antivenoms against animal envenomings, it is critical to have a thorough
159 understanding of venom complexity and which toxins to target. For this purpose, different omics
160 technologies are increasingly being used to uncover novel animal toxins and help guide antivenom
161 development.
162 To date, genomes have been reported for two snakes (King cobra, *Ophiophagus*
163 *Hannah*, and Burmese python, *Python molurus bivittatus*) [67,68], three spiders (African social
164 velvet spider, *Stegodyphus mimosarum*, Brazilian white-knee tarantula, *Acanthoscurria geniculate*,
165 and House spider, *Parasteatoda tepidariorum*) [69,70], one scorpion (Manchurian scorpion,
166 *Mesobuthus martensii*) [71], and the honey bee (*Apis mellifera*) [72]. In addition, a multitude of
167 transcriptomics studies has been performed, particularly on the venom glands from snakes,
168 scorpions, and spiders [8,9]. These have all provided important evolutionary insight into the biology
169 of venomous animals and may be used to uncover novel toxins with unique functionalities. In
170 general, however, correlation between mRNA transcripts and protein expression may not always be
171 high [73], which has on several occasions shown to be the case for certain snake venoms from both
172 the viper and elapid families [74–76]. Therefore, genomic and transcriptomic studies may benefit
173 from combination with proteomics to establish a full overview of a venom.
174 The state of the art for elucidation of venom composition is based on the venomics
175 approach, combining venom fractionation by chromatography and gel electrophoresis with mass
176 spectrometry (see Figure 1) [49,77,78]. These techniques can be used to quantitatively estimate

177 venom proteomes [79,80]. Venomics can be performed both by bottom-up [81] and top-down
178 approaches, with the latter having the benefit of being able to distinguish between closely related
179 toxin isoforms [82]. By mid-2015, approximately 140 snake venoms had undergone a venomics
180 analysis [8]. Since then, this number has been growing steadily with new studies on venomous
181 snakes from Asia, Africa, Australia, Europe, and Latin America [82–89]. Fewer venomics studies
182 have been performed on venoms from spiders, scorpions [9], bees, and insects, owing to the
183 amounts of venom needed for estimating quantitative venom proteomes, and the scarcity of venoms
184 from most of these rather small creatures. Therefore, most of the proteomic studies performed on
185 venoms from these smaller species are qualitative in nature and only provide a catalog of toxins
186 present in the venoms with unknown abundances.

187 Venomics provides a good descriptive overview of venom. However, to fully gain an
188 understanding of which toxins in a venom are important for an envenoming case, functional studies
189 are needed. One such approach is toxicovenomics (see Figure 1), which was introduced in 2015 in a
190 study of Black mamba (*Dendroaspis polylepis*) venom with the aim of identifying key toxins to be
191 neutralized by effective antivenom [90]. In this study, the Toxicity Score [11] was employed which
192 can be calculated for venom toxins and fractions (containing toxin mixtures) based on their
193 abundance and potency. The Toxicity Scores can be used to rank the toxins or fractions according
194 to their medical importance (typically judged by lethality in mice), although the difficulty of
195 isolating certain toxins, such as Snake Venom Metalloproteases (SVMPs) somewhat hinder its use
196 for some venoms [11]. Geographical and intraspecies variation may give rise to slightly different
197 estimates of quantitative venom proteomes, which may, in turn, affect the Toxicity Scores for toxins
198 in venom. Additionally, the presence of toxin synergism [91] in some snake venoms (such as the
199 Green mamba, *Dendroaspis angisticeps* [92]) may further obscure the identification of which toxins
200 are key neutralization targets. However, when used conservatively to select the toxins which are

essential to neutralize in order to abrogate overall venom toxicity, the toxicovenomics approach may provide a robust roadmap for antitoxin discovery. So far only elapid snake species from the *Naja, Dendroaspis*, and *Aipysurus* genera have been investigated by toxicovenomics [18,90,92–94]. However, researchers from Mexico have recently employed a similar approach for selecting which toxins from Mexican scorpions to focus antibody discovery efforts on [53].

Understanding cross-reactivity by antivenomics and high-density peptide microarray technology

In addition to employing proteomics tools to gain a more complete understanding of whole venoms and which toxins to neutralize with novel antivenom components, it may be beneficial to harness omics technologies to assess antivenoms and antitoxins to guide their development. A particularly important feature for toxin-neutralizing components is their ability to (selectively) cross-neutralize entire (sub)families of venom toxins, as this allows for a decreased number of antitoxins needed in a novel antivenom. Traditionally, most studies focusing on cross-reactivity and cross-neutralization have been based on ELISA, enzymatic *in vitro* assays, and *in vivo* rodent assays. However, new technologies emerging within the antibody field allow for more holistic and high-throughput assessments of cross-reactivity.

One of the main issues with assessing antibodies by ELISA is that cross-reactivity often correlates poorly with cross-neutralization. As a solution to this, "antivenomics" has been developed based on the same methodology as venomics [95,96]. In the antivenomics approach, whole venom is pre-incubated with beads coated with antivenom antibodies, before both bound and unbound venom components are analyzed by Reversed-Phase High-Performance Liquid Chromatography (RP-HPLC) and compared with the RP-HPLC chromatogram and proteomic analysis of the whole venom (see Figure 2) [97]. This approach, which has so far only been used for

serum-based antivenoms, allows for a holistic assessment of the binding capacity of antivenoms against different venom components, which may further be used to assess (potential) cross-reactivity to toxins from other snake species that the antivenom was not raised against [78,96]. Nevertheless, the methodology should, in theory, be just as useful for assessing cross-reactivity of mAbs. Antivenomic studies have been performed on antivenoms against a range of different snake species from Europe, Asia, Oceania, and Latin America [87,98–104]. They are yet to be performed on non-snake species, possibly owing to the scarcity of venoms from smaller venomous animals.

To obtain a more detailed molecular view of cross-reactivity, immunoreactivity of antivenoms has also been evaluated using synthetic linear peptides derived from amino acid sequences of spider, scorpion, and snake toxins [105–109]. Generally, these studies have been quite meticulous and low throughput, but recently a novel high-throughput approach was introduced based on high-density peptide microarray technology (see Figure 3) [110]. In this pioneering study, the cross-reactivity of three different antivenoms against sub-Saharan African snake species was assessed and the linear elements of epitopes for all 82 reported neurotoxins from the *Dendroaspis* and *Naja* genera mapped simultaneously in one experiment. Although both the previous approaches and the more recent high-throughput techniques have so far only been employed in the assessment of serum-based antivenoms, their applicability to characterization of mAbs is known from other fields [111,112].

The feasibility of assessing both monoclonal and oligoclonal antibodies using both antivenomics and high-density peptide microarrays may possibly be instrumental for *in vitro* assessment of novel recombinant antivenoms, to reduce cost, guide development, and reduce the amount of preclinical work needed.

Conclusion

Envenomings by venomous animals are one of the therapeutic areas, where medicines based on animal sera are still in use. Different approaches have been tested and reported in the pursuit of developing fundamentally novel antivenoms. These approaches have involved design of novel immunogens (synthetic epitope DNA strings, synthetic linear peptides, and recombinant toxins and toxids), the use of small molecule inhibitors against enzymatic toxins, isolation and preclinical testing of endogenous animal proteins with toxin-neutralizing capabilities, and various recombinant antibody formats. Other binding proteins exist that could be harnessed, but these are yet to be employed within antivenom development. The most promising approach is likely to be the use of mixtures of (human or camelid) mAbs due to their versatility, high target affinity, low immunogenicity, and decreasing cost of production.

Irrespective of the approach employed, antivenom development efforts may benefit significantly by being guided by different omics technologies. Genomics and transcriptomics are excellent tools for uncovering novel animal toxins and investigating venom evolution. However, the state of the art remains to be venom proteomics (particularly venomics and toxicovenomics), which provides a better overview of venom compositions and what toxin targets are essential to neutralize in envenoming cases. Finally, the use of antivenomics and high-density peptide microarray technology may be exploited to gain in-depth understanding of antivenom and antibody specificity and cross-reactivity.

Acknowledgements

Thanks go to Mia Øhlenschlæger for proof-reading.

Funding

272 This work was supported by the Novo Nordisk Foundation (NNF16OC0019248) for financial
273 support.

References

[1] Gutiérrez JM, Williams D, Fan HW, Warrell DA. Snakebite envenoming from a global perspective: Towards an integrated approach. Toxicon 2010;56:1223–35. doi:10.1016/j.toxicon.2009.11.020.

[2] Harrison RA, Cook DA, Renjifo C, Casewell NR, Currier RB, Wagstaff SC. Research strategies to improve snakebite treatment: Challenges and progress. J Proteomics 2011;74:1768–80. doi:10.1016/j.jprot.2011.06.019.

[3] Harrison RA, Gutiérrez JM. Priority Actions and Progress to Substantially and Sustainably Reduce the Mortality, Morbidity and Socioeconomic Burden of Tropical Snakebite. Toxins 2016;8. doi:10.3390/toxins8120351.

[4] David Warrell, José María Gutiérrez, A. Padilla. Rabies and Envenomings: a Neglected Public Health Issue: Report of a Consultative Meeting. Geneva: World Health Organization; 2007.

[5] Diaz JH. The Global Epidemiology, Syndromic Classification, Management, and Prevention of Spider Bites. Am J Trop Med Hyg 2004;71:239–50.

[6] Carmo AO, Chatzaki M, Horta CCR, Magalhães BF, Oliveira-Mendes BBR, Chávez-Olórtegui C, et al. Evolution of alternative methodologies of scorpion antivenoms production. Toxicon 2015;97:64–74. doi:10.1016/j.toxicon.2015.02.007.

[7] Fatima Laraba-Djebari, Sonia Adi-Bessalem, Djelila Hammoudi-Triki. Scorpion Venoms: Pathogenesis and Biotherapies. Toxinology - Scorpion Venoms, vol. 4, Dordrecht Heidelberg New York London: Springer; 2015, p. 580.

[8] Laustsen AH, Engmark M, Milbo C, Johannesen J, Lomonte B, Gutiérrez JM, et al. From Fangs to Pharmacology: The Future of Snakebite Envenoming Therapy. Curr Pharm Des 2016;22:5270–93.

[9] Laustsen AH, Solà M, Jappe EC, Oscoz S, Lauridsen LP, Engmark M. Biotechnological Trends in Spider and Scorpion Antivenom Development. Toxins 2016;8:226. doi:10.3390/toxins8080226.

[10] Gutiérrez JM, León G, Lomonte B, Angulo Y. Antivenoms for snakebite envenomings. Inflamm Allergy Drug Targets 2011;10:369–80.

[11] Laustsen AH, Lohse B, Lomonte B, Engmark M, Gutiérrez JM. Selecting key toxins for focused development of elapid snake antivenoms and inhibitors guided by a Toxicity Score. Toxicon 2015;104:43–5. doi:10.1016/j.toxicon.2015.07.334.

[12] Calvete JJ. Proteomic tools against the neglected pathology of snake bite envenoming. Expert Rev Proteomics 2011;8:739–58. doi:10.1586/epr.11.61.

[13] Calvete JJ, Lomonte B. A bright future for integrative venomics. Toxicon 2015;107, Part B:159–62. doi:10.1016/j.toxicon.2015.10.024.

[14] Warrell DA. Clinical toxicology of snakebite in Asia. In: Meier J, White J, editors. Handb. Clin. Toxicol. Anim. Venoms Poisons, Boca Raton: CRC Press; 1995, p. 493–594.

[15] Warrell DA. Snakebites in Central and South America: Epidemiology, clinical features, and clinical management. In: Brodie III ED, editor. Venom. Reptil. West. Hemisphere, Ithaca, NY: Cornell University Press; 2004, p. 709–61.

[16] Laustsen AH, Engmark M, Clouser C, Timberlake S, Vigneault F, Gutiérrez JM, et al. Exploration of immunoglobulin transcriptomes from mice immunized with three-finger

toxins and phospholipases A2 from the Central American coral snake, Micrurus nigrocinctus. PeerJ 2017;5:e2924. doi:10.7717/peerj.2924.
[17] Alape-Girón A, Sanz L, Escolano J, Flores-Díaz M, Madrigal M, Sasa M, et al. Snake Venomics of the Lancehead Pitviper *Bothrops asper* : Geographic, Individual, and Ontogenetic Variations. J Proteome Res 2008;7:3556–71. doi:10.1021/pr800332p.
[18] Laustsen AH, Gutiérrez JM, Rasmussen AR, Engmark M, Gravlund P, Sanders KL, et al. Danger in the reef: Proteome, toxicity, and neutralization of the venom of the olive sea snake, Aipysurus laevis. Toxicon 2015;107, Part B:187–96. doi:10.1016/j.toxicon.2015.07.008.
[19] Wagstaff SC, Laing GD, Theakston RDG, Papaspyridis C, Harrison RA. Bioinformatics and Multiepitope DNA Immunization to Design Rational Snake Antivenom. PLoS Med 2006;5:e209. doi:10.1371/journal.pmed.0030184.
[20] Calderon-Aranda ES, Selisko B, York EJ, Gurrola GB, Stewart JM, Possani LD. Mapping of an epitope recognized by a neutralizing monoclonal antibody specific to toxin Cn2 from the scorpion Centruroides noxius, using discontinuous synthetic peptides. Eur J Biochem 1999;264:746–55. doi:10.1046/j.1432-1327.1999.00620.x.
[21] Guatimosim SCF, Kalapothakis E, Diniz CR, Chávez-Olórtegui C. Induction of neutralizing antibodies against Tityus serrulatus toxins by immunization with a recombinant nontoxic protein. Toxicon 2000;38:113–21. doi:10.1016/S0041-0101(99)00138-5.
[22] Lewin M, Samuel S, Merkel J, Bickler P. Varespladib (LY315920) Appears to Be a Potent, Broad-Spectrum, Inhibitor of Snake Venom Phospholipase A2 and a Possible Pre-Referral Treatment for Envenomation. Toxins 2016;8. doi:10.3390/toxins8090248.
[23] Howes J-M, Theakston RDG, Laing GD. Neutralization of the haemorrhagic activities of viperine snake venoms and venom metalloproteinases using synthetic peptide inhibitors and chelators. Toxicon Off J Int Soc Toxinology 2007;49:734–9. doi:10.1016/j.toxicon.2006.11.020.
[24] Feng L, Gao R, Gopalakrishnakone P. Isolation and characterization of a hyaluronidase from the venom of Chinese red scorpion Buthus martensi. Comp Biochem Physiol Part C Toxicol Pharmacol 2008;148:250–7. doi:10.1016/j.cbpc.2008.06.003.
[25] Morey SS, Kiran KM, Gadag JR. Purification and properties of hyaluronidase from Palamneus gravimanus (Indian black scorpion) venom. Toxicon 2006;47:188–95. doi:10.1016/j.toxicon.2005.10.014.
[26] Ramanaiah, M., Parthasarathy, Pr., Venkaiah, B. Isolation and characterization of hyaluronidase from scorpion (Heterometrus fulvipes) venom. Biochem Int 1989;20:301–10.
[27] Soares AM, Ticli FK, Marcussi S, Lourenço MV, Januário AH, Sampaio SV, et al. Medicinal plants with inhibitory properties against snake venoms. Curr Med Chem 2005;12:2625–41.
[28] Trento EP, Garcia OS, Rucavado A, França SC, Batalini C, Arantes EC, et al. Inhibitory properties of the anti-bothropic complex from Didelphis albiventris serum on toxic and pharmacological actions of metalloproteases and myotoxins from Bothrops asper venom. Biochem Pharmacol 2001;62:1521–9.
[29] Neves-Ferreira AGC, Perales J, Fox JW, Shannon JD, Makino DL, Garratt RC, et al. Structural and functional analyses of DM43, a snake venom metalloproteinase inhibitor from Didelphis marsupialis serum. J Biol Chem 2002;277:13129–37. doi:10.1074/jbc.M200589200.
[30] Neves-Ferreira AG, Perales J, Ovadia M, Moussatché H, Domont GB. Inhibitory properties of the antibothropic complex from the South American opossum (Didelphis marsupialis) serum. Toxicon Off J Int Soc Toxinology 1997;35:849–63.

[31] Borkow G, Gutierrez JM, Ovadia M. A potent antihemorrhagin in the serum of the non-poisonous water snake Natrix tessellata: isolation, characterization and mechanism of neutralization. Biochim Biophys Acta 1994;1201:482–90.
[32] Lizano S, Lomonte B, Fox JW, Gutiérrez JM. Biochemical characterization and pharmacological properties of a phospholipase A2 myotoxin inhibitor from the plasma of the snake Bothrops asper. Biochem J 1997;326 (Pt 3):853–9.
[33] Borkow G, Gutierrez JM, Ovadia M. Isolation, characterization and mode of neutralization of a potent antihemorrhagic factor from the serum of the snake Bothrops asper. Biochim Biophys Acta 1995;1245:232–8.
[34] Lomonte B, Cerdas L, Gené JA, Gutiérrez JM. Neutralization of local effects of the terciopelo (Bothrops asper) venom by blood serum of the colubrid snake Clelia clelia. Toxicon Off J Int Soc Toxinology 1982;20:571–9.
[35] Thwin MM, Gopalakrishnakone P. Snake envenomation and protective natural endogenous proteins: a mini review of the recent developments (1991-1997). Toxicon Off J Int Soc Toxinology 1998;36:1471–82.
[36] Biardi JE, Coss RG, Smith DG. California ground squirrel (Spermophilus beecheyi) blood sera inhibits crotalid venom proteolytic activity. Toxicon Off J Int Soc Toxinology 2000;38:713–21.
[37] Stumpp MT, Binz HK, Amstutz P. DARPins: a new generation of protein therapeutics. Drug Discov Today 2008;13:695–701. doi:10.1016/j.drudis.2008.04.013.
[38] Rasool M, Malik A, Hussain M, Haq KA, Butt K, Basit A, et al. DARPins Bioengineering and its Theranostic Approaches: Emerging Trends in Protein Engineering. Curr Pharm Des 2016.
[39] Varadamsetty G, Tremmel D, Hansen S, Parmeggiani F, Plückthun A. Designed Armadillo repeat proteins: library generation, characterization and selection of peptide binders with high specificity. J Mol Biol 2012;424:68–87. doi:10.1016/j.jmb.2012.08.029.
[40] Béhar G, Renodon-Cornière A, Mouratou B, Pecorari F. Affitins as robust tailored reagents for affinity chromatography purification of antibodies and non-immunoglobulin proteins. J Chromatogr A 2016;1441:44–51. doi:10.1016/j.chroma.2016.02.068.
[41] Pacheco S, Béhar G, Maillasson M, Mouratou B, Pecorari F. Affinity transfer to the archaeal extremophilic Sac7d protein by insertion of a CDR. Protein Eng Des Sel PEDS 2014;27:431–8. doi:10.1093/protein/gzu042.
[42] Correa A, Pacheco S, Mechaly AE, Obal G, Béhar G, Mouratou B, et al. Potent and Specific Inhibition of Glycosidases by Small Artificial Binding Proteins (Affitins). PLoS ONE 2014;9. doi:10.1371/journal.pone.0097438.
[43] Tiede C, Tang AAS, Deacon SE, Mandal U, Nettleship JE, Owen RL, et al. Adhiron: a stable and versatile peptide display scaffold for molecular recognition applications. Protein Eng Des Sel PEDS 2014;27:145–55. doi:10.1093/protein/gzu007.
[44] Schiefner A, Skerra A. The menagerie of human lipocalins: a natural protein scaffold for molecular recognition of physiological compounds. Acc Chem Res 2015;48:976–85. doi:10.1021/ar5003973.
[45] Simeon R, Chen Z. In vitro-engineered non-antibody protein therapeutics. Protein Cell 2017. doi:10.1007/s13238-017-0386-6.
[46] Pessenda G, Silva LC, Campos LB, Pacello EM, Pucca MB, Martinez EZ, et al. Human scFv antibodies (Afribumabs) against Africanized bee venom: Advances in melittin recognition. Toxicon 2016;112:59–67. doi:10.1016/j.toxicon.2016.01.062.

[47] Boulain JC, Ménez A, Couderc J, Faure G, Liacopoulos P, Fromageot P. Neutralizing monoclonal antibody specific for Naja nigricollis toxin alpha: preparation, characterization, and localization of the antigenic binding site. Biochemistry (Mosc) 1982;21:2910–5.
[48] Roncolato EC, Campos LB, Pessenda G, Costa e Silva L, Furtado GP, Barbosa JE. Phage display as a novel promising antivenom therapy: A review. Toxicon 2015;93:79–84. doi:10.1016/j.toxicon.2014.11.001.
[49] Laustsen AH. Recombinant antivenoms. 1st ed. Copenhagen, Denmark: University of Copenhagen; 2016.
[50] Pucca MB, Zoccal KF, Roncolato EC, Bertolini TB, Campos LB, Cologna CT, et al. Serrumab: A human monoclonal antibody that counters the biochemical and immunological effects of Tityus serrulatus venom. J Immunotoxicol 2012;9:173–83. doi:10.3109/1547691X.2011.649220.
[51] Pucca MB, Cerni FA, Peigneur S, Arantes EC, Tytgat J, Barbosa JE. Serrumab: A novel human single chain-fragment antibody with multiple scorpion toxin-neutralizing capacities. J Immunotoxicol 2014;11:133–40. doi:10.3109/1547691X.2013.809175.
[52] Roncolato EC, Pucca MB, Funayama JC, Bertolini TB, Campos LB, Barbosa JE. Human antibody fragments specific for Bothrops jararacussu venom reduce the toxicity of other Bothrops sp. venoms. J Immunotoxicol 2013;10:160–8. doi:10.3109/1547691X.2012.703253.
[53] Rodríguez-Rodríguez ER, Olamendi-Portugal T, Serrano-Posada H, Arredondo-López JN, Gómez-Ramírez I, Fernández-Taboada G, et al. Broadening the neutralizing capacity of a family of antibody fragments against different toxins from Mexican scorpions. Toxicon 2016;119:52–63. doi:10.1016/j.toxicon.2016.05.011.
[54] Rodríguez-Rodríguez ER, Ledezma-Candanoza LM, Contreras-Ferrat LG, Olamendi-Portugal T, Possani LD, Becerril B, et al. A Single Mutation in Framework 2 of the Heavy Variable Domain Improves the Properties of a Diabody and a Related Single-Chain Antibody. J Mol Biol 2012;423:337–50. doi:10.1016/j.jmb.2012.07.007.
[55] di Tommaso A, Juste MO, Martin-Eauclaire M-F, Dimier-Poisson I, Billiald P, Aubrey N. Diabody Mixture Providing Full Protection against Experimental Scorpion Envenoming with Crude Androctonus australis Venom. J Biol Chem 2012;287:14149–56. doi:10.1074/jbc.M112.348912.
[56] Abderrazek RB, Hmila I, Vincke C, Benlasfar Z, Pellis M, Dabbek H, et al. Identification of potent nanobodies to neutralize the most poisonous polypeptide from scorpion venom. Biochem J 2009;424:263–72. doi:10.1042/BJ20090697.
[57] Hmila I, Abdallah R BA-B, Saerens D, Benlasfar Z, Conrath K, Ayeb ME, et al. VHH, bivalent domains and chimeric Heavy chain-only antibodies with high neutralizing efficacy for scorpion toxin AahI′. Mol Immunol 2008;45:3847–56. doi:10.1016/j.molimm.2008.04.011.
[58] Hmila I, Cosyns B, Tounsi H, Roosens B, Caveliers V, Abderrazek RB, et al. Pre-clinical studies of toxin-specific Nanobodies: Evidence of in vivo efficacy to prevent fatal disturbances provoked by scorpion envenoming. Toxicol Appl Pharmacol 2012;264:222–31. doi:10.1016/j.taap.2012.07.033.
[59] Stewart CS, MacKenzie CR, Hall JC. Isolation, characterization and pentamerization of alpha-cobrotoxin specific single-domain antibodies from a naïve phage display library: preliminary findings for antivenom development. Toxicon Off J Int Soc Toxinology 2007;49:699–709. doi:10.1016/j.toxicon.2006.11.023.
[60] Chavanayarn C, Thanongsaksrikul J, Thueng-in K, Bangphoomi K, Sookrung N, Chaicumpa W. Humanized-Single Domain Antibodies (VH/VHH) that Bound Specifically to Naja

459 kaouthia Phospholipase A2 and Neutralized the Enzymatic Activity. Toxins 2012;4:554–67.
460 doi:10.3390/toxins4070554.
461 [61] Hmila I, Saerens D, Abderrazek RB, Vincke C, Abidi N, Benlasfar Z, et al. A bispecific
462 nanobody to provide full protection against lethal scorpion envenoming. FASEB J
463 2010;24:3479–89. doi:10.1096/fj.09-148213.
464 [62] Richard G, Meyers AJ, McLean MD, Arbabi-Ghahroudi M, MacKenzie R, Hall JC. In Vivo
465 Neutralization of α-Cobratoxin with High-Affinity Llama Single-Domain Antibodies (VHHs)
466 and a VHH-Fc Antibody. PLoS ONE 2013;8:e69495. doi:10.1371/journal.pone.0069495.
467 [63] Ringel M, Tollman P, Hersch G, Schulze U. Does size matter in R&D productivity? If not,
468 what does? Nat Rev Drug Discov 2013;12:901–2. doi:10.1038/nrd4164.
469 [64] Laustsen AH, Johansen KH, Engmark M, Andersen MR. Snakebites: costing recombinant
470 antivenoms. Nature 2016;538:41. doi:10.1038/538041e.
471 [65] Laustsen AH, Johansen KH, Engmark M, Andersen MR. Recombinant snakebite antivenoms:
472 A cost-competitive solution to a neglected tropical disease? PLoS Negl Trop Dis
473 2017;11:e0005361. doi:10.1371/journal.pntd.0005361.
474 [66] Kelley B. Very large scale monoclonal antibody purification: the case for conventional unit
475 operations. Biotechnol Prog 2007;23:995–1008. doi:10.1021/bp070117s.
476 [67] Vonk FJ, Casewell NR, Henkel CV, Heimberg AM, Jansen HJ, McCleary RJR, et al. The
477 king cobra genome reveals dynamic gene evolution and adaptation in the snake venom
478 system. Proc Natl Acad Sci 2013;110:20651–6. doi:10.1073/pnas.1314702110.
479 [68] Castoe TA, Koning APJ de, Hall KT, Card DC, Schield DR, Fujita MK, et al. The Burmese
480 python genome reveals the molecular basis for extreme adaptation in snakes. Proc Natl Acad
481 Sci 2013;110:20645–50. doi:10.1073/pnas.1314475110.
482 [69] Sanggaard KW, Bechsgaard JS, Fang X, Duan J, Dyrlund TF, Gupta V, et al. Spider genomes
483 provide insight into composition and evolution of venom and silk. Nat Commun 2014;5.
484 doi:10.1038/ncomms4765.
485 [70] Gendreau KL, Haney RA, Schwager EE, Wierschin T, Stanke M, Richards S, et al. House
486 spider genome uncovers evolutionary shifts in the diversity and expression of black widow
487 venom proteins associated with extreme toxicity. BMC Genomics 2017;18:178.
488 doi:10.1186/s12864-017-3551-7.
489 [71] Cao Z, Yu Y, Wu Y, Hao P, Di Z, He Y, et al. The genome of Mesobuthus martensii reveals
490 a unique adaptation model of arthropods. Nat Commun 2013;4:2602.
491 doi:10.1038/ncomms3602.
492 [72] Beye M, Gattermeier I, Hasselmann M, Gempe T, Schioett M, Baines JF, et al. Exceptionally
493 high levels of recombination across the honey bee genome. Genome Res 2006;16:1339–44.
494 doi:10.1101/gr.5680406.
495 [73] Haider S, Pal R. Integrated Analysis of Transcriptomic and Proteomic Data. Curr Genomics
496 2013;14:91–110. doi:10.2174/1389202911314020003.
497 [74] Durban J, Juárez P, Angulo Y, Lomonte B, Flores-Diaz M, Alape-Girón A, et al. Profiling
498 the venom gland transcriptomes of Costa Rican snakes by 454 pyrosequencing. BMC
499 Genomics 2011;12:259. doi:10.1186/1471-2164-12-259.
500 [75] Tan CH, Tan KY, Fung SY, Tan NH. Venom-gland transcriptome and venom proteome of
501 the Malaysian king cobra (Ophiophagus hannah). BMC Genomics 2015;16:687.
502 doi:10.1186/s12864-015-1828-2.
503 [76] Tan KY, Tan CH, Chanhome L, Tan NH. Comparative venom gland transcriptomics of Naja
504 kaouthia (monocled cobra) from Malaysia and Thailand: elucidating geographical venom
505 variation and insights into sequence novelty. PeerJ 2017;5:e3142. doi:10.7717/peerj.3142.

506 [77] Calvete JJ. Next-generation snake venomics: protein-locus resolution through venom
507 proteome decomplexation. Expert Rev Proteomics 2014;11:315–29.
508 doi:10.1586/14789450.2014.900447.
509 [78] Calvete JJ. Snake venomics: From the inventory of toxins to biology. Toxicon 2013;75:44–
510 62. doi:10.1016/j.toxicon.2013.03.020.
511 [79] Gibbs HL, Sanz L, Calvete JJ. Snake population venomics: proteomics-based analyses of
512 individual variation reveals significant gene regulation effects on venom protein expression
513 in Sistrurus rattlesnakes. J Mol Evol 2009;68:113–25. doi:10.1007/s00239-008-9186-1.
514 [80] Gutiérrez JM, Lomonte B, León G, Alape-Girón A, Flores-Díaz M, Sanz L, et al. Snake
515 venomics and antivenomics: Proteomic tools in the design and control of antivenoms for the
516 treatment of snakebite envenoming. J Proteomics 2009;72:165–82.
517 doi:10.1016/j.jprot.2009.01.008.
518 [81] Warrell DA, Gutiérrez JM, Calvete JJ, Williams D. New approaches & technologies of
519 venomics to meet the challenge of human envenoming by snakebites in India. Indian J Med
520 Res 2013;138:38–59.
521 [82] Petras D, Heiss P, Harrison RA, Süssmuth RD, Calvete JJ. Top-down venomics of the East
522 African green mamba, Dendroaspis angusticeps, and the black mamba, Dendroaspis
523 polylepis, highlight the complexity of their toxin arsenals. J Proteomics 2016;146:148–64.
524 doi:10.1016/j.jprot.2016.06.018.
525 [83] Tan CH, Tan KY, Yap MKK, Tan NH. Venomics of Tropidolaemus wagleri, the sexually
526 dimorphic temple pit viper: Unveiling a deeply conserved atypical toxin arsenal. Sci Rep
527 2017;7:43237. doi:10.1038/srep43237.
528 [84] Latinović Z, Leonardi A, Šribar J, Sajevic T, Žužek MC, Frangež R, et al. Venomics of
529 Vipera berus berus to explain differences in pathology elicited by Vipera ammodytes
530 ammodytes envenomation: Therapeutic implications. J Proteomics 2016;146:34–47.
531 doi:10.1016/j.jprot.2016.06.020.
532 [85] Tan NH, Wong KY, Tan CH. Venomics of Naja sputatrix, the Javan spitting cobra: A short
533 neurotoxin-driven venom needing improved antivenom neutralization. J Proteomics
534 2017;157:18–32. doi:10.1016/j.jprot.2017.01.018.
535 [86] Tang ELH, Tan CH, Fung SY, Tan NH. Venomics of Calloselasma rhodostoma, the Malayan
536 pit viper: A complex toxin arsenal unraveled. J Proteomics 2016;148:44–56.
537 doi:10.1016/j.jprot.2016.07.006.
538 [87] Gay C, Sanz L, Calvete JJ, Pla D. Snake Venomics and Antivenomics of Bothrops diporus, a
539 Medically Important Pitviper in Northeastern Argentina. Toxins 2015;8.
540 doi:10.3390/toxins8010009.
541 [88] Tan CH, Fung SY, Yap MKK, Leong PK, Liew JL, Tan NH. Unveiling the elusive and
542 exotic: Venomics of the Malayan blue coral snake (Calliophis bivirgata flaviceps). J
543 Proteomics 2016;132:1–12. doi:10.1016/j.jprot.2015.11.014.
544 [89] Reeks T, Lavergne V, Sunagar K, Jones A, Undheim E, Dunstan N, et al. Deep venomics of
545 the Pseudonaja genus reveals inter- and intra-specific variation. J Proteomics 2016;133:20–
546 32. doi:10.1016/j.jprot.2015.11.019.
547 [90] Laustsen AH, Lomonte B, Lohse B, Fernández J, Gutiérrez JM. Unveiling the nature of black
548 mamba (Dendroaspis polylepis) venom through venomics and antivenom immunoprofiling:
549 Identification of key toxin targets for antivenom development. J Proteomics 2015;119:126–
550 42. doi:10.1016/j.jprot.2015.02.002.
551 [91] Laustsen AH. Toxin synergism in snake venoms. Toxin Rev 2016;35:165–70.
552 doi:10.1080/15569543.2016.1220397.

[92] Lauridsen LP, Laustsen AH, Lomonte B, Gutiérrez JM. Toxicovenomics and antivenom profiling of the Eastern green mamba snake (Dendroaspis angusticeps). J Proteomics 2016;136:248–61. doi:10.1016/j.jprot.2016.02.003.
[93] Lauridsen LP, Laustsen AH, Lomonte B, Gutiérrez JM. Exploring the venom of the forest cobra snake: Toxicovenomics and antivenom profiling of Naja melanoleuca. J Proteomics 2016;150:98–108. doi:10.1016/j.jprot.2016.08.024.
[94] Laustsen AH, Gutiérrez JM, Lohse B, Rasmussen AR, Fernández J, Milbo C, et al. Snake venomics of monocled cobra (Naja kaouthia) and investigation of human IgG response against venom toxins. Toxicon 2015;99:23–35. doi:10.1016/j.toxicon.2015.03.001.
[95] Calvete JJ. Antivenomics and venom phenotyping: A marriage of convenience to address the performance and range of clinical use of antivenoms. Toxicon 2010;56:1284–91. doi:10.1016/j.toxicon.2009.12.015.
[96] Gutiérrez JM, Lomonte B, Sanz L, Calvete JJ, Pla D. Immunological profile of antivenoms: Preclinical analysis of the efficacy of a polyspecific antivenom through antivenomics and neutralization assays. J Proteomics 2014;105:340–50. doi:10.1016/j.jprot.2014.02.021.
[97] Calvete JJ, Sanz L, Angulo Y, Lomonte B, Gutiérrez JM. Venoms, venomics, antivenomics. FEBS Lett 2009;583:1736–43. doi:10.1016/j.febslet.2009.03.029.
[98] Calvete JJ, Cid P, Sanz L, Segura álvaro, Villalta M, Herrera M, et al. Antivenomic Assessment of the Immunological Reactivity of EchiTAb-Plus-ICP, an Antivenom for the Treatment of Snakebite Envenoming in Sub-Saharan Africa. Am J Trop Med Hyg 2010;82:1194–201. doi:10.4269/ajtmh.2010.09-0733.
[99] Xu N, Zhao H-Y, Yin Y, Shen S-S, Shan L-L, Chen C-X, et al. Combined venomics, antivenomics and venom gland transcriptome analysis of the monocoled cobra (Naja kaouthia) from China. J Proteomics 2017. doi:10.1016/j.jprot.2017.02.018.
[100] Calvete JJ, Arias AS, Rodríguez Y, Quesada-Bernat S, Sánchez LV, Chippaux JP, et al. Preclinical evaluation of three polyspecific antivenoms against the venom of Echis ocellatus: Neutralization of toxic activities and antivenomics. Toxicon Off J Int Soc Toxinology 2016;119:280–8. doi:10.1016/j.toxicon.2016.06.022.
[101] Goncalves-Machado L. Combined venomics, venom gland transcriptomics, bioactivities, and antivenomics of two Bothrops jararaca populations from geographic isolated regions within the Brazilian Atlantic rainforest. J Proteomics 2016;135:73–89. doi:10.1016/j.jprot.2015.04.029.
[102] Pla D, Bande BW, Welton RE, Paiva OK, Sanz L, Segura Á, et al. Proteomics and antivenomics of Papuan black snake (Pseudechis papuanus) venom with analysis of its toxicological profile and the preclinical efficacy of Australian antivenoms. J Proteomics 2017;150:201–15. doi:10.1016/j.jprot.2016.09.007.
[103] Pla D, Calvete JJ, Elosua C, Paniagua J. Antivenomics of a European vipera antivenom. Toxicon 2016;119:371. doi:10.1016/j.toxicon.2016.06.025.
[104] Pla D, Calvete JJ, Elosua C, Paniagua J. Antivenomics of a sub-saharan Africa antivenom antivipmyn Africa. Toxicon 2016;119:371–2. doi:10.1016/j.toxicon.2016.06.026.
[105] De-Simone SG, Napoleão-Pego P, Teixeira-Pinto LAL, Santos JDL, De-Simone TS, Melgarejo AR, et al. Linear B-cell epitopes in BthTX-1, BthTX-II and BthA-1, phospholipase A2's from Bothrops jararacussu snake venom, recognized by therapeutically neutralizing commercial horse antivenom. Toxicon 2013;72:90–101. doi:10.1016/j.toxicon.2013.06.004.
[106] Lomonte B. Identification of linear B-cell epitopes on myotoxin II, a Lys49 phospholipase A2 homologue from Bothrops asper snake venom. Toxicon 2012;60:782–90. doi:10.1016/j.toxicon.2012.05.028.

[107] Castro KL, Duarte CG, Ramos HR, Machado de Avila RA, Schneider FS, Oliveira D, et al. Identification and characterization of B-cell epitopes of 3FTx and PLA2 toxins from Micrurus corallinus snake venom. Toxicon 2015;93:51–60. doi:10.1016/j.toxicon.2014.10.015.
[108] Maria WS, Velarde DT, Alvarenga LM, Nguyen C, Villard S, Granier C, et al. Localization of epitopes in the toxins of Tityus serrulatus scorpions and neutralizing potential of therapeutic antivenoms. Toxicon 2005;46:210–7. doi:10.1016/j.toxicon.2005.03.024.
[109] Ramada JS, Becker-Finco A, Minozzo JC, Felicori LF, Machado de Avila RA, Molina F, et al. Synthetic peptides for in vitro evaluation of the neutralizing potency of Loxosceles antivenoms. Toxicon 2013;73:47–55. doi:10.1016/j.toxicon.2013.07.007.
[110] Engmark M, Andersen MR, Laustsen AH, Patel J, Sullivan E, Masi F de, et al. High-throughput immuno-profiling of mamba (Dendroaspis) venom toxin epitopes using high-density peptide microarrays. Sci Rep 2016;6:36629. doi:10.1038/srep36629.
[111] Halperin RF, Stafford P, Johnston SA. Exploring Antibody Recognition of Sequence Space through Random-Sequence Peptide Microarrays. Mol Cell Proteomics MCP 2011;10. doi:10.1074/mcp.M110.000786.
[112] Price JV, Tangsombatvisit S, Xu G, Levy D, Baechler EC, Gozani O, et al. “On silico” peptide microarrays for high-resolution mapping of antibody epitopes and diverse protein-protein interactions. Nat Med 2012;18:1434–40. doi:10.1038/nm.2913.

Figure legends

Figure 1. *Schematic overview of venomics and toxicovenomics. Following the venomics approach, venoms are fractionated by HPLC and gel electrophoresis, and the different fractions are enzymatically digested and analyzed by MALDI-TOF-TOF and bioinformatics for identification of toxins. Using chromatographic data and SDS-PAGE densitometry, it is possible to quantitatively estimate the venom proteome. Toxicovenomics builds upon venomics and includes in vivo toxicity data, which can be used to obtain an overview of venoms as pharmacological targets for antitoxin development and determine which toxins are essential to neutralize with antivenom in an envenoming case.*

Figure 2. *Schematic overview of the antivenomics approach.* ***A)*** *First venom is passed through a column containing resins that are coated with antivenom. Unbound toxins are analyzed by Reversed-Phase High-Performance Liquid Chromatography (RP-HPLC).* ***B)*** *Bound venom toxins are then eluted and likewise analyzed by RP-HPLC.* ***C)*** *Combined with the chromatogram and a proteomic analysis of the whole venom, the two analyses provide an overview of which venom toxins are readily recognized and bound by a given antivenom, and which toxins are not.*

Figure 3. *Schematic overview of how high-density peptide microarrays are designed and used for the study of epitope-paratope interactions between antivenoms and animal venom toxins.*

Highlights

- nvenoming by venomous animals remain a major public health challenge in the tropics
- Biotechnological approaches are being harnessed for development of novel antivenoms
- Genomics and transcriptomics are good for uncovering novel venom toxins
- Venom proteomics is useful for identifying key toxins for neutralization by novel antivenoms
- High-density peptide microarray technology is emerging as a valuable tool in antivenom research