

## High-throughput epitope identification for snakebite antivenom

**Engmark, Mikael; De Masi, Federico; Laustsen, Andreas Hougaard; Gutiérrez, José María; Lomonte, Bruno; Andersen, Mikael Rørdam; Lund, Ole**

*Publication date:*  
2015

*Document Version*  
Publisher's PDF, also known as Version of record

[Link back to DTU Orbit](#)

*Citation (APA):*  
Engmark, M., De Masi, F., Laustsen, A. H., Gutiérrez, J. M., Lomonte, B., Andersen, M. R., & Lund, O. (2015). High-throughput epitope identification for snakebite antivenom. Poster session presented at The International Sustainability Conference 2015, Falmer, Brighton, United Kingdom.

## DTU Library

Technical Information Center of Denmark

---

### General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.



# High-throughput epitope identification for snakebite antivenom

Mikael Engmark<sup>1</sup>, Federico De Masi<sup>1</sup>, Andreas Hougaard Laustsen<sup>2</sup>, José María Gutiérrez<sup>3</sup>, Bruno Lomonte<sup>3</sup>, Mikael Rørdam Andersen<sup>1</sup>, and Ole Lund<sup>1</sup>

(1) Department of Systems Biology, Technical University of Denmark  
(2) Department of Drug Design and Pharmacology, Faculty of Health and Medical Sciences, University of Copenhagen  
(3) Instituto Clodomiro Picado, Facultad de Microbiología, Universidad de Costa Rica  
Correspondence: [miken@bio.dtu.dk](mailto:miken@bio.dtu.dk)

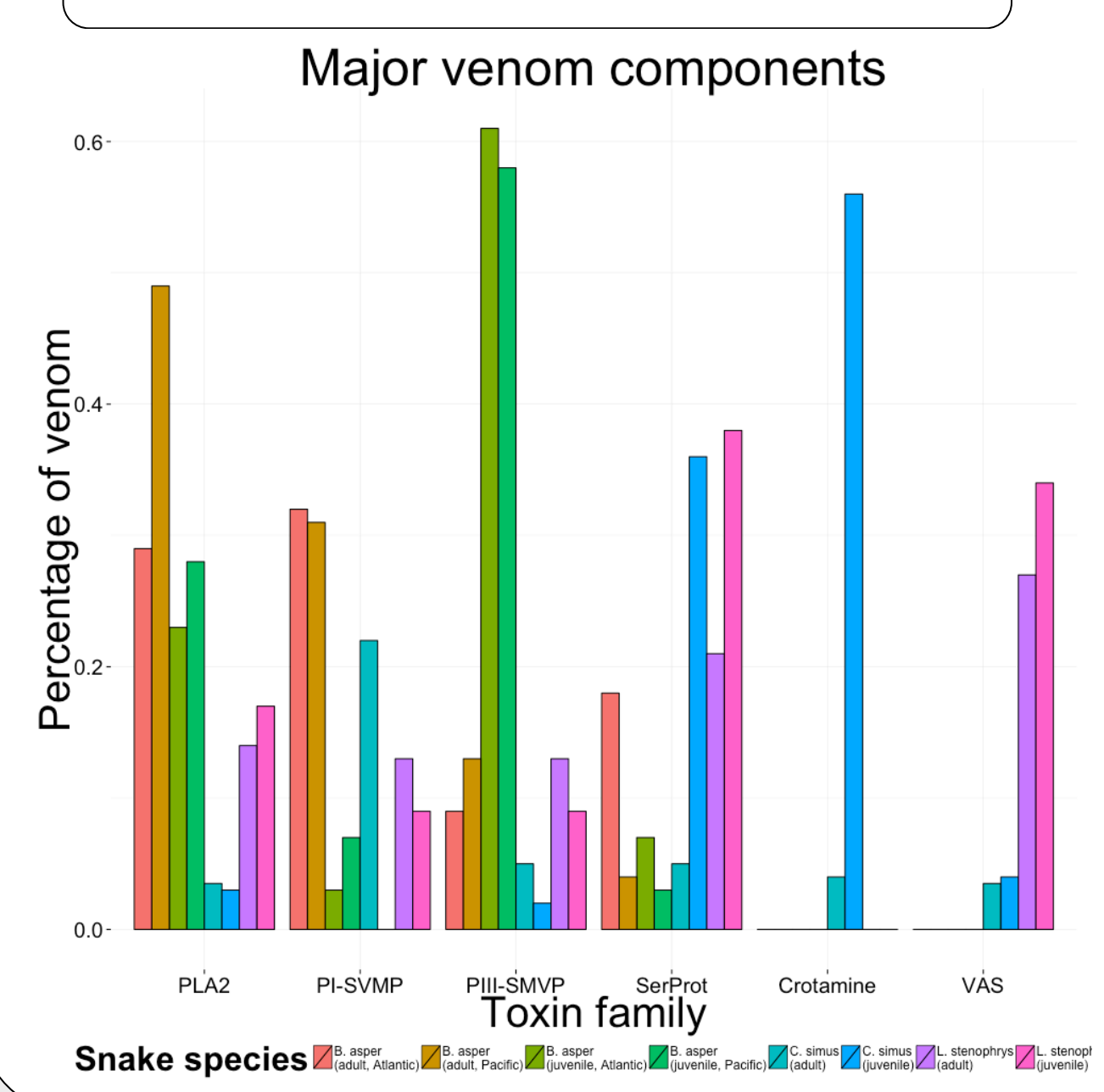
## Introduction

Insight into the epitopic recognition pattern for polyclonal antivenoms is a strong tool for accurate prediction of antivenom cross-reactivity and provides a basis for design of novel antivenoms. In this work, a high-throughput approach was applied to characterize linear epitopes in 966 individual toxins from pit vipers (Crotalidae) using the ICP Crotalidae antivenom. Due to an abundance of snake venom metalloproteinases and phospholipase A<sub>2</sub>s in the venoms used for production of the investigated antivenom, this study focuses on these toxin families.

### Objectives

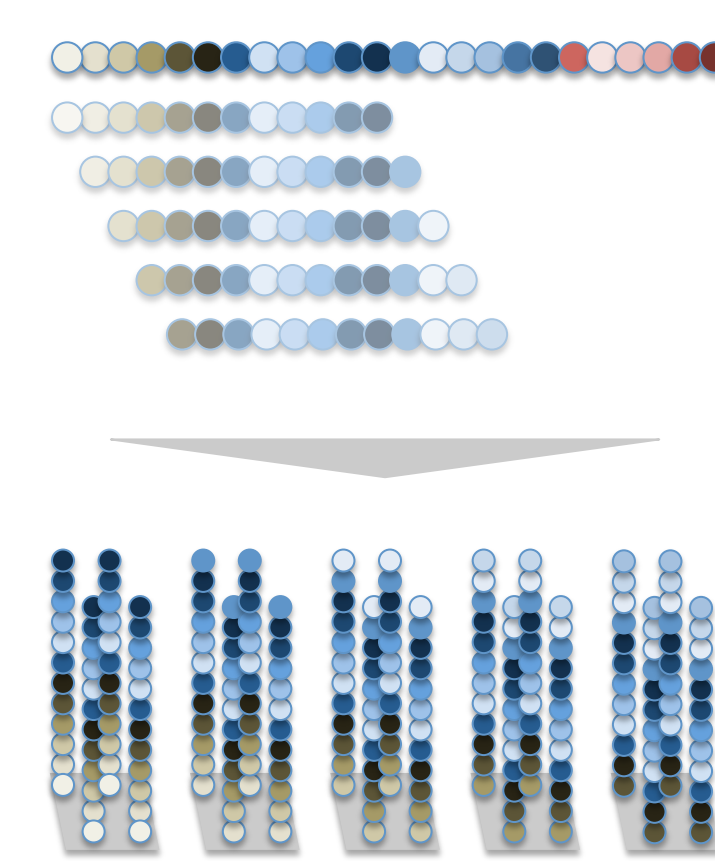
- Identify epitopes in toxins used in immunization
- Characterize tolerated amino acid substitutions in identified epitopes
- Predict cross-reactivity of antivenom

### Immunization mixture<sup>1</sup>



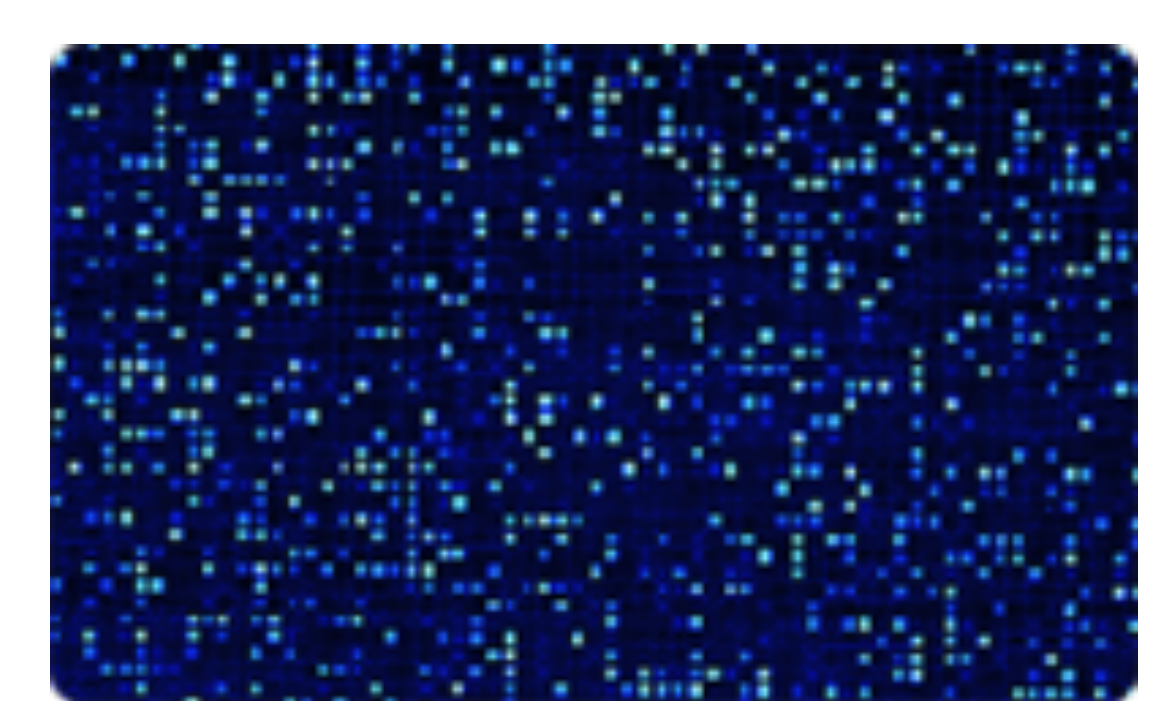
### Studying linear epitopes using peptide microarrays

*In silico* generation of peptide library



Synthesis on microarray

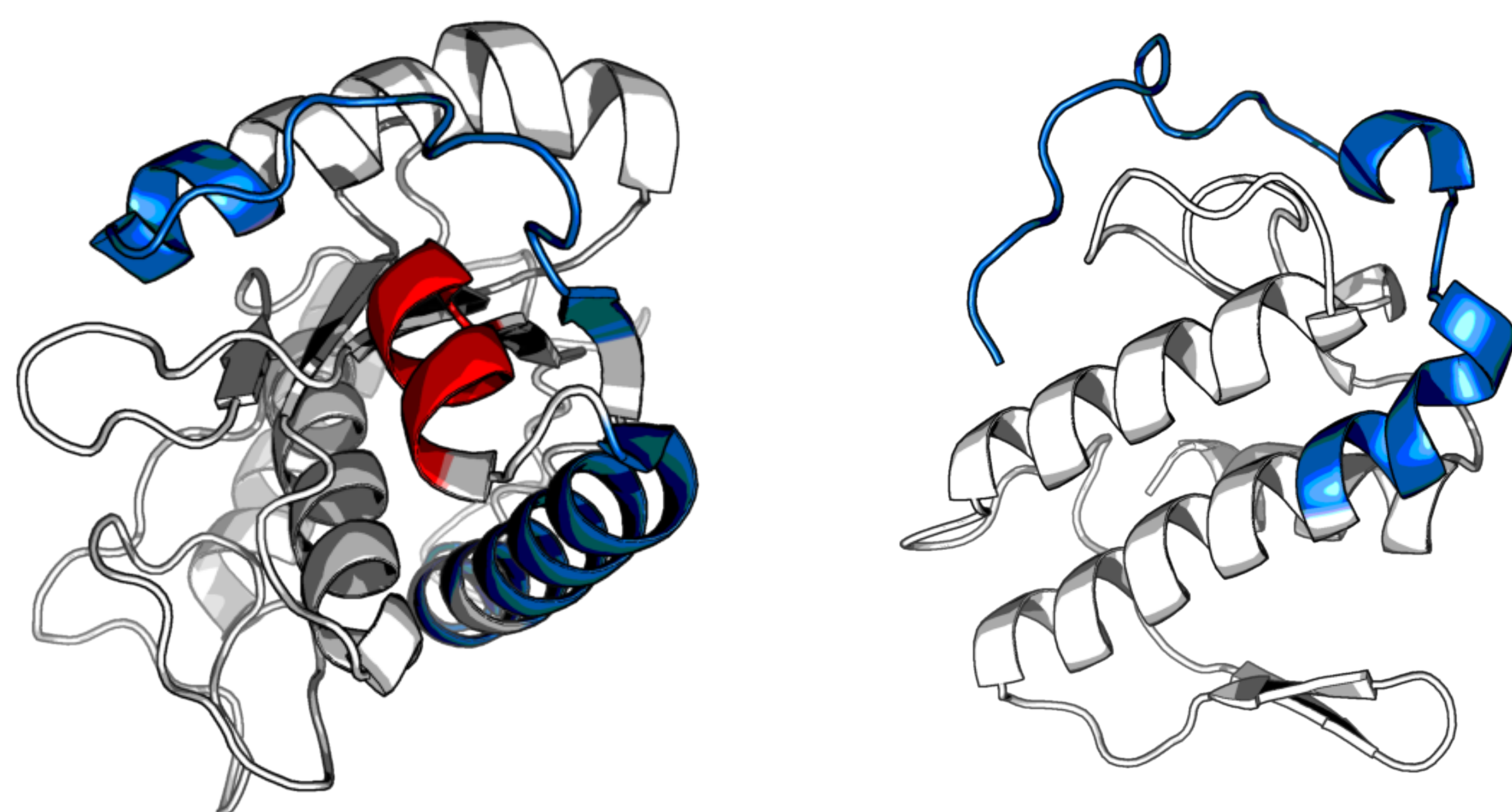
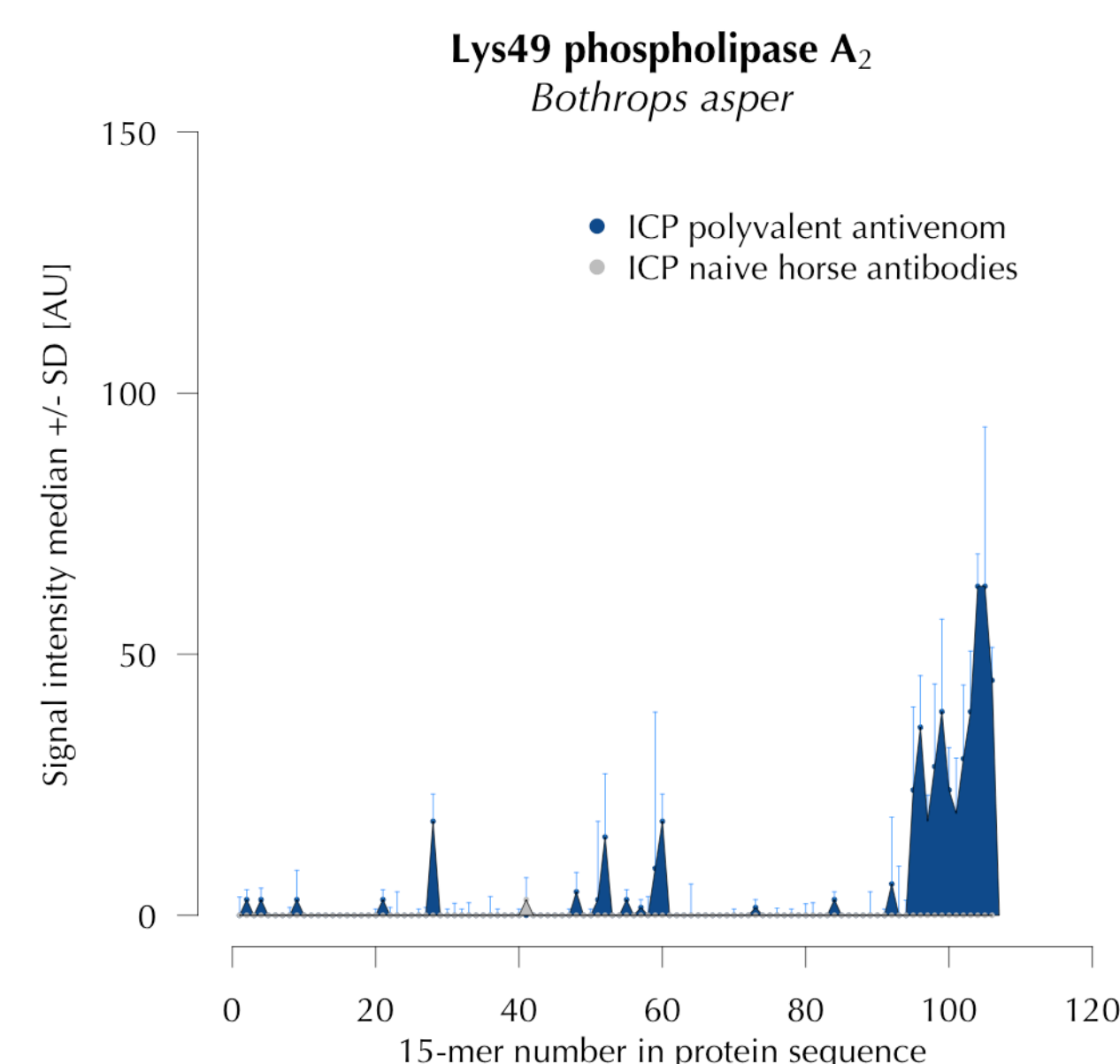
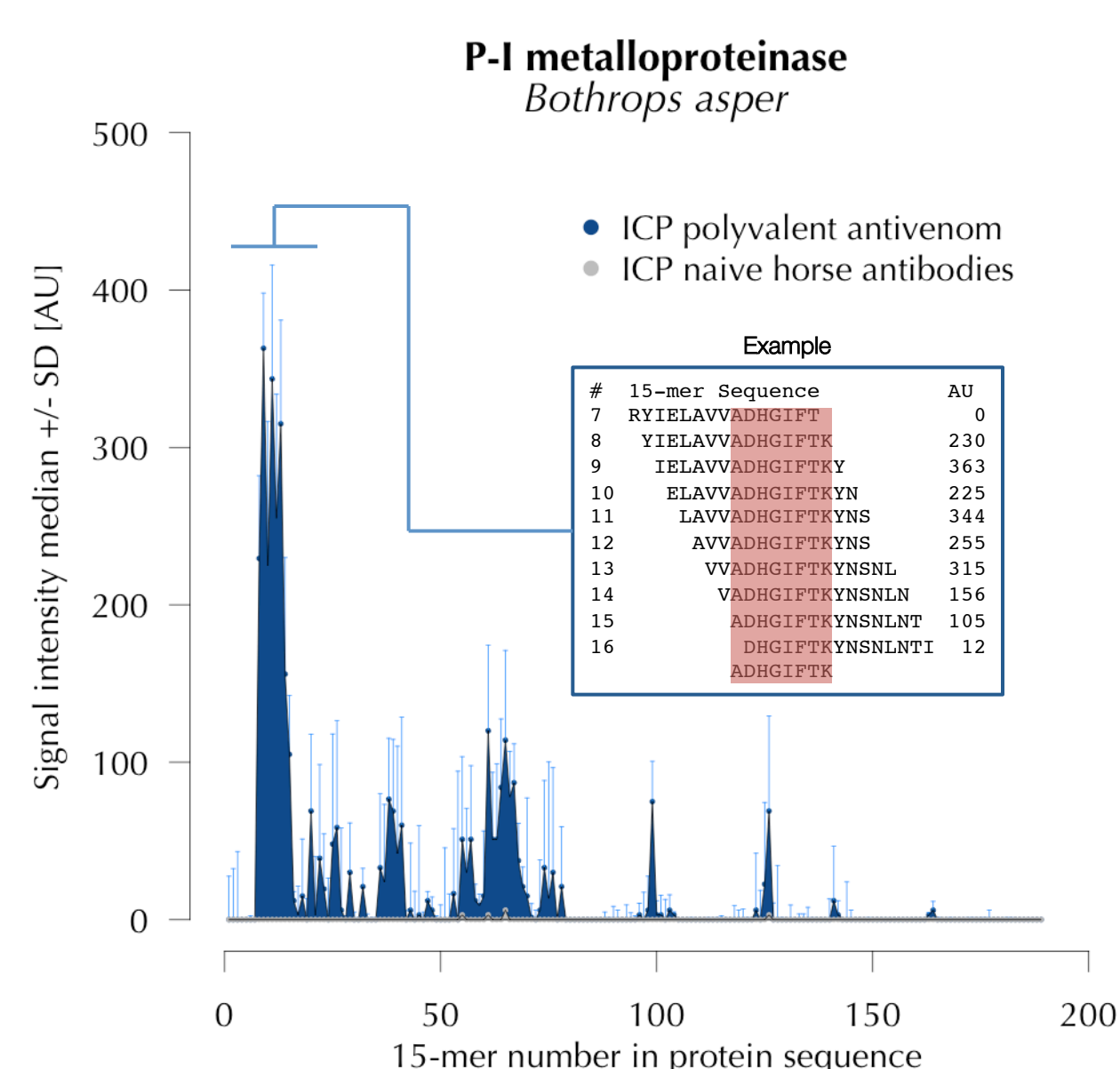
Antibody binding and detection



Data analysis and protein modeling

### Epitopes locate to surface regions

To identify epitopes the observed peptide specific signal intensities were mapped back to the amino acid sequence of each pit viper toxin. Using two or more overlapping 15-mer peptides with median signals above 20 AU, epitope core sequences were localized and subsequently mapped to crystal structures or homology models. As examples, P-I metalloproteinase and Lys49-phospholipase A<sub>2</sub> from *Bothrops asper* (venom used in antivenom production) are presented here.



The epitope core sequences are highlighted in blue except for the high-signal epitope in *Bothrops asper* P-I metalloproteinase that is highlighted in red. All epitopes are found to be exposed on the protein surface. The structure of the metalloproteinase (PDB: 2W13)<sup>2</sup> was obtained from the Protein Data Bank (pdb.org), and the homology model of the phospholipase A<sub>2</sub> was built using CPHmodels<sup>3</sup> based on a crystal structure of the Lys49-phospholipase from *B. moojeni* (PDB: 4KF3)<sup>4</sup> with 87.7% identity.

### Effect on cross-recognition

CLUSTAL O(1.2.1) multiple sequence alignment

QUERY		-----ADHGIFTK-----	Mean AU overlapping peptides
Q072L5	<i>Bothrops asper</i>	YIELAVVADHGIFTKYNSNLTNTI	249.0
P83512	<i>Bothrops asper</i>	YIELAVVADHGIFTKYNSNLTNTI	249.0
P0DJE1	<i>Bothrops asper</i>	YIELAVVADHGIFTKYNSNLTNTI	249.0
Q5XUW8	<i>Bothrops insularis</i>	YIELAVVADHGMFTKYNSNLTNTI	189.9
E3UJL4	<i>Bothrops neuwiedi</i>	YIELAVVADHGMFTKYNSNVNTI	232.8
P0C6S0	<i>Bothrops pauloensis</i>	YIELAVVADHGMFTKYNSNNTI	188.3
C0HJU2	<i>Bothrops pauloensis</i>	YIELAVVADHGMFTKYNSNVNTI	232.8
P0C6S1	<i>Bothrops pauloensis</i>	YIELAVVADHGMFTKYNSNIDTI	187.1
P22796	<i>Lachesis muta</i>	YIELVVADHGMFTKYNGNLNTI	191.1
T1DJY5	<i>Crotalus horridus</i>	YVELVIVADHGMFTKYNGNLKKI	187.5
J3SBQ2	<i>Crotalus adamanteus</i>	YVELVIVADHGMFTKYNRNLTEV	174.2
J3SBQ1	<i>Crotalus adamanteus</i>	YVELVIVADHGMFTKYNRNLTEV	174.2
J3RY86	<i>Crotalus adamanteus</i>	YVELVIVADHGMFTKYNRNLTEV	174.2
F8S112	<i>Crotalus adamanteus</i>	YVELVIVADHGMFTKYNRNLTEV	174.2
O73795	<i>Gloydus brevicaudus</i>	YIELVIVADHGMFTKYNGSDKI	200.3
Q90WC0	<i>Gloydus brevicaudus</i>	YIELVIVADHGMFTKYNGSDKI	200.3
Q698K8	<i>Gloydus brevicaudus</i>	YIELVIVADHGMFTKYNGSDKI	200.3
Q1PBD1	<i>Gloydus halys</i>	YIELVIVADHGMFTKYNSNLDTI	154.9
J3S830	<i>Crotalus adamanteus</i>	YVELVIVADHGMFTKYNRNLTEV	174.2
Q9YI19	<i>Gloydus brevicaudus</i>	YIELVVADHGMFTKYNSNLDTI	164.4
Q9PVK9	<i>Gloydus brevicaudus</i>	YIELVVADHGMFTKYNSNLDTI	164.4

The  $\alpha$ -helix shaped red epitope in the *B. asper* metalloproteinase is found to be highly conserved among pit viper metalloproteinases. Based on multiple sequence alignment of pit viper toxins sharing at least seven of the eight epitope residues and mean signal intensity of the eight 15-mer peptides harboring the epitope, we find that flanking residues outside of the core epitope has small effect on antivenom recognition. Expanding the analysis to the 42 toxins that share at least five of the epitope residues, binding is still observed in all of the corresponding eight 15-mer peptides, although the microarray signals are reduced up to seven times (data not shown).

These results suggest that ICP Crotalidae polyvalent antivenom might offer protection from the investigated metalloproteinases, including the toxins from the Asian *Gloydus* species if these *in vitro* experiments translate to the *in vivo* situation.

### Conclusions

- Custom-designed high density peptide microarray technology enables parallel automated identification of epitopes in hundreds of toxins
- Integrating multiple sequence alignment allows investigation of the effect of epitope variation on antivenom recognition
- Cross-reactivity of antivenom is correlated to the degree of conservation in toxin epitopes and flanking residues

### References

- Gutiérrez JM, et al. *Journal of Proteomics*. 2014; 105: 340–350. doi:10.1016/j.jprot.2014.02.021
- Lingott T, et al. *Biochemistry*. 2009; 48: 6166–6174. doi:10.1021/bi9002315
- Nielsen M, et al. *Nucleic Acids Research*. 2010; 38: 576–581. doi:10.1093/nar/gkq535
- Salvador GHM, et al. *Toxicon*. 2013; 72: 52–63. doi:10.1016/j.toxicon.2013.06.013

### Acknowledgement

The peptide microarray experiments were performed at Schafer-N, Copenhagen. We would like to thank Claus Schafer, Christian Skjødt Hansen, and Jens Kringelum for experimental setup and support. We further thank the Novo Nordisk Foundation for financial support (grant number: NNF13OC0005613)