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Discovery of Human IgGs against α-Cobratoxin for Development of Recombinant Antibody-based Antivenom

Andreas Hougaard Laustsen[1], Mikael Engmark[2], Arne Redsted Rasmussen[3], and Brian Lohse[1]

[1]Department of Drug Design and Pharmacology, University of Copenhagen, [2]Department of Systems Biology, Technical University of Denmark, [3]Royal Danish Academy of Fine Arts Schools of Architecture, Design and Conservation,

Snakebite – A Neglected Tropical Disease

More than 5.5 million people are bitten by venomous snakes per year on a global basis. This leads to approx. 125,000 deaths and 3 times as many amputations [1]. Particularly Sub-Saharan Africa is affected by the problem [2], [3]. Current antivenoms are still being produced by a method developed in the 1890's, in which large mammals (typically horses) are immunized with snake venom and antiserum is derived from the animals blood. The incompatibility with the human immune system of these animal derived antivenoms leads to a range of side effects, such as serum sickness, anaphylaxis, and sometimes even death [2]. Despite the maturity of medicinal chemistry and advances in drug development, there remains a need for modern antivenoms with better safety profile and improved efficacy [4].

We have set out to tackle this challenge by attempting to develop the World's first antivenom based on recombinant, humanized antibodies. Such an antivenom will be cheaper to produce in large scale and is anticipated to have a much improved safety and efficacy profile.
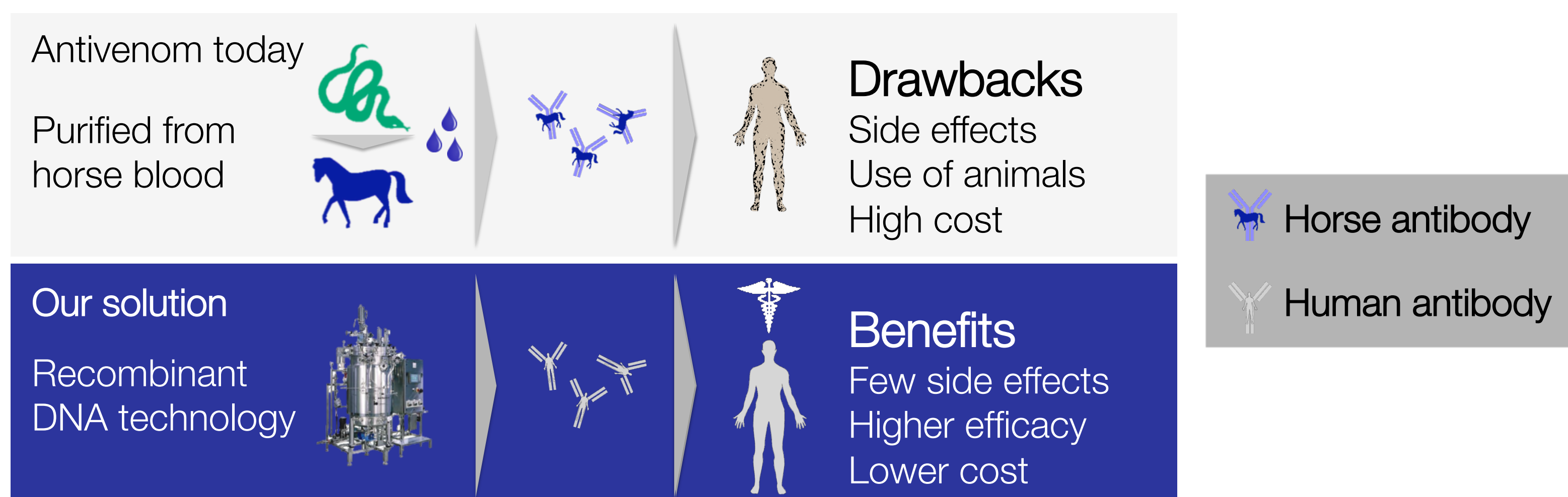

Figure 1: Overview of the current production process for antivenoms and our proposed solution employing recombinantly expressed humanized antibodies.

Methods – Human IgGs Isolated from Human Serum

Serum was obtained from a human donor who had repeatedly injected himself with small amounts of snake venom from a range of different snakes, among others *Naja kaouthia* (Thai cobra). From the donor serum, polyclonal IgG antibodies were isolated and purified by employing a Protein A Antibody Purification Kit. To assess the presence of IgG antibodies directed against cobra venom toxins, an ELISA assay was performed. Detailed experimental procedures are is described elsewhere [5].

A bioinformatics analysis was performed, which included visual comparison of the two snake toxins α-cobratoxin (PDB entry: 4AEA) and dendrotoxin I (PDB entry: 1DEM) and sequence alignment using the protein ClustalW2 algorithm from EMBL-EBI.
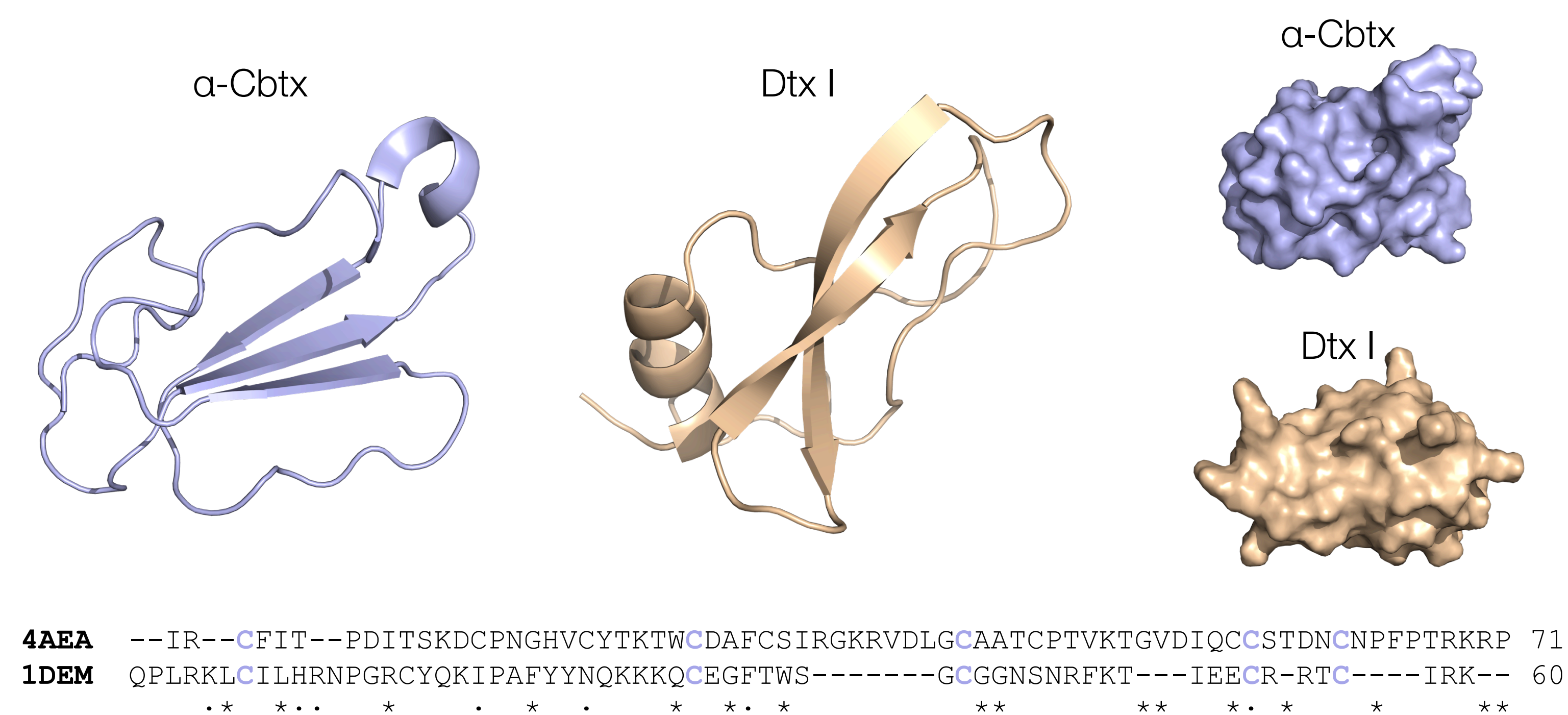


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4AEA  --IR--CFIT--PDITSKDCPNGHVCYTKTWCDAFCSIRGKRVDLGCAATCPTVKTGVDIQCCSTDNCNPFPTRKRP 71
1DEM  QPLRKLCILHRNPGRCYQKIPAFYYNQKKKQCEGFTWS-------GCGGNSNRFKT---IEECR-RTC----IRK-- 60
        :*  *::  *.  :.* * :. .*. *:.*      **....** *:.* .*.  **
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Figure 2: Cartoon models and surface models drawn in PyMOL of α-cobratoxin (α-Cbtx, PDB entry: 4AEA) and dendrotoxin I (Dtx I, PDB entry: 1DEM) and sequence alignment performed using the protein ClustalW2 algorithm from EMBL-EBI. Low sequence similarity of 29.63% is observed and the secondary and tertiary structures are quite dissimilar.
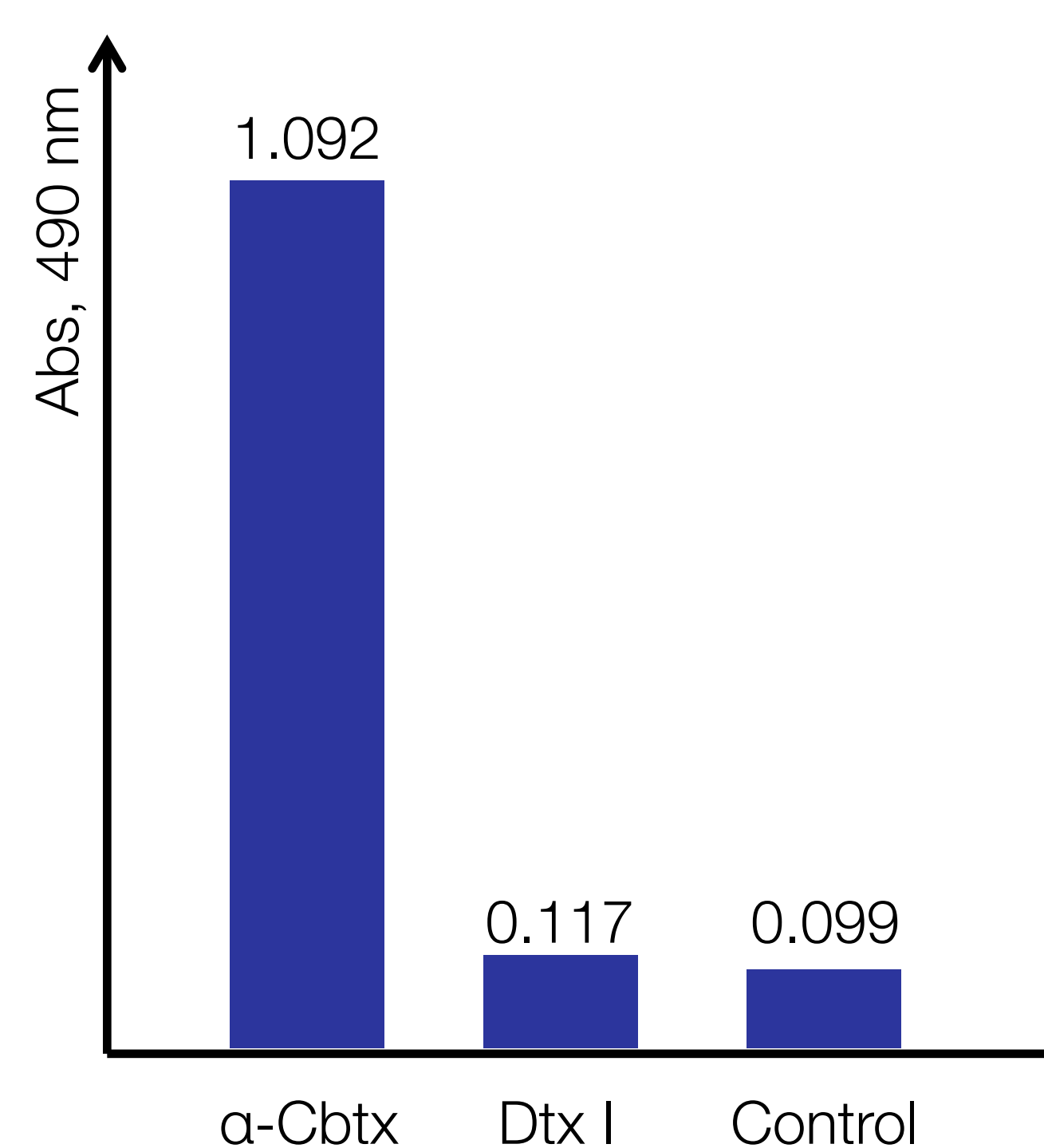

Figure 3: ELISA experiment. A purified polyclonal mixture of IgG antibodies from a human donor that had injected himself with venom from *Naja kaouthia* (Thai cobra), were tested against α-cobratoxin (α-Cbtx) from *Naja kaouthia*, Dendrotoxin I (Dtx I) from *Dendroaspis polylepis* (Black Mamba), and a control (PBS). A strong IgG response is found for the IgGs against α-Cbtx, but not Dtx I, indicating that the administration of *Naja kaouthia* venom has induced an immune response against the neurotoxic α-Cbtx, but not Dtx I. The donor had never administered venom from *Dendroaspis polylepis*, and the lack of IgG response against Dtx I was therefore to be expected.

Results – α-Cobratoxin and Dendrotoxin I Dissimilar
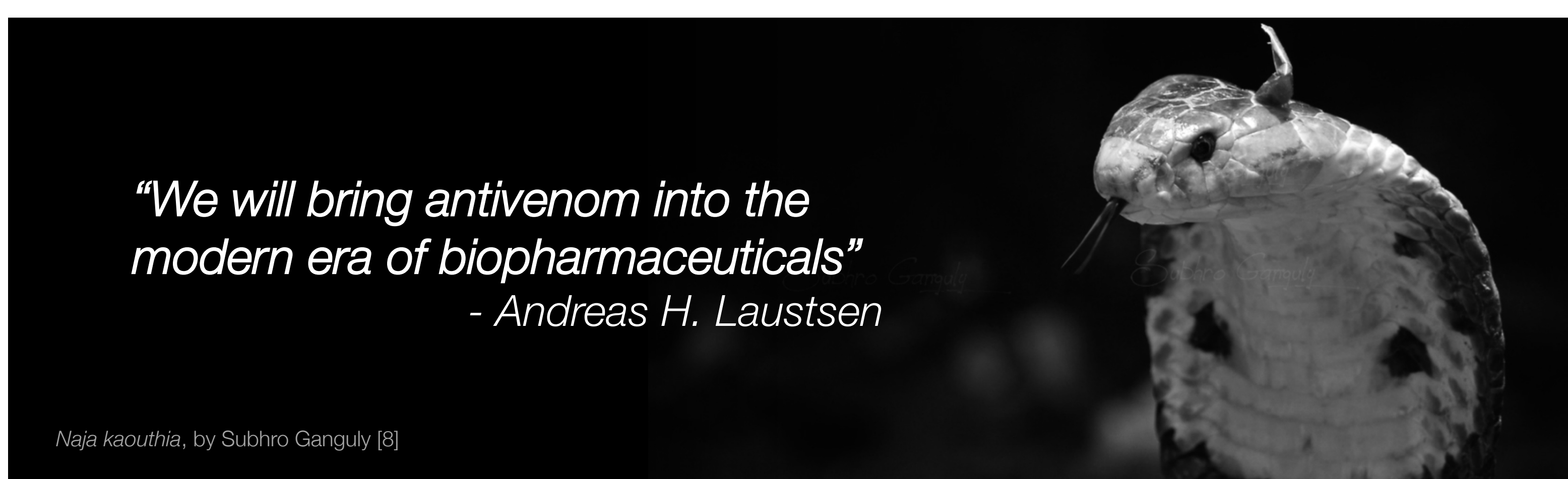
In the bioinformatics analysis it was shown that the similarity between α-cobratoxin and dendrotoxin I was a low 29.63% (see figure 2). This result was unsurprising given that the two neurotoxins have different targets (Nicotinic Acetylcholine Receptor [6], and K+ channel [7]).

In the ELISA assay it was shown that the isolated and purified human IgG antibodies had a strong response to α-cobratoxin, but not to dendrotoxin I (see figure 3).

Discussion – Cross-reactivity not easily Attainable

Human IgG antibodies induced by immunological challenge with venom from *Naja kaouthia* show reactivity against α-cobratoxin from the same snake species, but not against Dendrotoxin I from *Dendroaspis polylepis*. This is unsurprising, since α-cobratoxin and dendrotoxin I were found to be significantly different both in terms of sequence similarity and tertiary structure.

IgG cross-reactivity against toxins from different snake species cannot generally be obtained. Antivenom is most likely to have efficacy in a given snakebite case if the antivenom is derived from a mammal that has been immunized with venom from the same (or perhaps in some cases: closely related) snake as the snakebite victim has been bitten by.

"We will bring antivenom into the modern era of biopharmaceuticals"
- *Andreas H. Laustsen*

Naja kaouthia, by Subhro Ganguly [8]

Future Perspectives – Using FACS for IgG Discovery

Our goal now is to isolate the single-most potent IgG antibody against α-cobratoxin from the polyclonal IgG pool and test it as an antitoxin candidate. Two strategies will be pursued (the second as a backup) in order to obtain the genetic sequence for the IgG antibody:

• Isolation of the relevant B-lymphocyte expressing the potent IgG antibody through Fluorescence Activated Cell Sorting (FACS) using fluorophore-conjugated α-cobratoxin follllowedby amplification of the correct genes and DNA sequencing.
• Immunoprecipitation of the IgG antibody using beads conjugated with α-cobratoxin followed by *de novo* sequencing by mass spectrometry.

This may pave the way for the World's first biopharmaceutical antivenom.

References
[1] Williams, D. J. et al. (2011). Ending the drought: New strategies for improving the flow of affordable, effective antivenoms in Asia and Africa. *Journal of Proteomics*, 74(9), 1735–1767.
[2] Gutiérrez, J. M. et al. (2010). Snakebite envenoming from a global perspective: Towards an integrated approach. *Toxicon*, 56(7), 1223–1235.
[3] Brown, N. I. (2012). Consequences of neglect: Analysis of the sub-saharan african snake antivenom market and the global context. *PLoS Neglected Tropical Diseases*, 6(6), e1670.
[4] Laustsen, A. et al. (2014). From Fangs to Pharmacology. *Article in drafting.*
[5] Laustsen, A. et al. (TBD). Discovery of snake toxin binders through phage display. *Unpublished results.*
[6] http://pdb.org/pdb/explore/explore.do?structureId=4aea
[7] http://pdb.org/pdb/explore/explore.do?structureId=1dem
[8] Borrowed with permission: https://www.flickr.com/photos/subhroclicks/7696661576/in/photolist-ebSVmY-7iTAz6-mgNTU3-
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