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Development of a Recombinant Antibody-Based Treatment of Snakebites

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Improving Antivenom to Save Lives and Limbs

Antivenom for snakebites is produced by immunization of large mammals with snake venom using a traditional and expensive method developed in the 1890's. Due to the animal origin, the products are highly immunogenic and come with a high risk of adverse side effects such as serum sickness and anaphylaxis, possibly leading to death [1].

This project aims at replacing existing snake antivenoms with a mixture of recombinant, humanized antibodies produced by modern cell-based fermentation technology [2]. It is anticipated that such an antivenom will reduce the current high risk of severe side effects, reduce cost, and thereby can be sold at 1/10 of the current price making the essential medicine available for > 700 M Africans [3].

Modern day technology allows development of monoclonal antibodies (mAbs) targeting snake toxins, however, identification, characterization of immunogenic features (B-cell epitopes), and availability of purified snake toxins or non-toxic analogs currently constitute major bottlenecks blocking the development of recombinant mAbs. We have set out to remove these bottlenecks starting by mapping antibody binding sites of existing horse-derived products and purified antibodies from snakebite victims using high-density peptide microarrays. Moreover, we are developing homology models of all relevant mamba toxins to map conserved sites and identify key residues for toxicity.

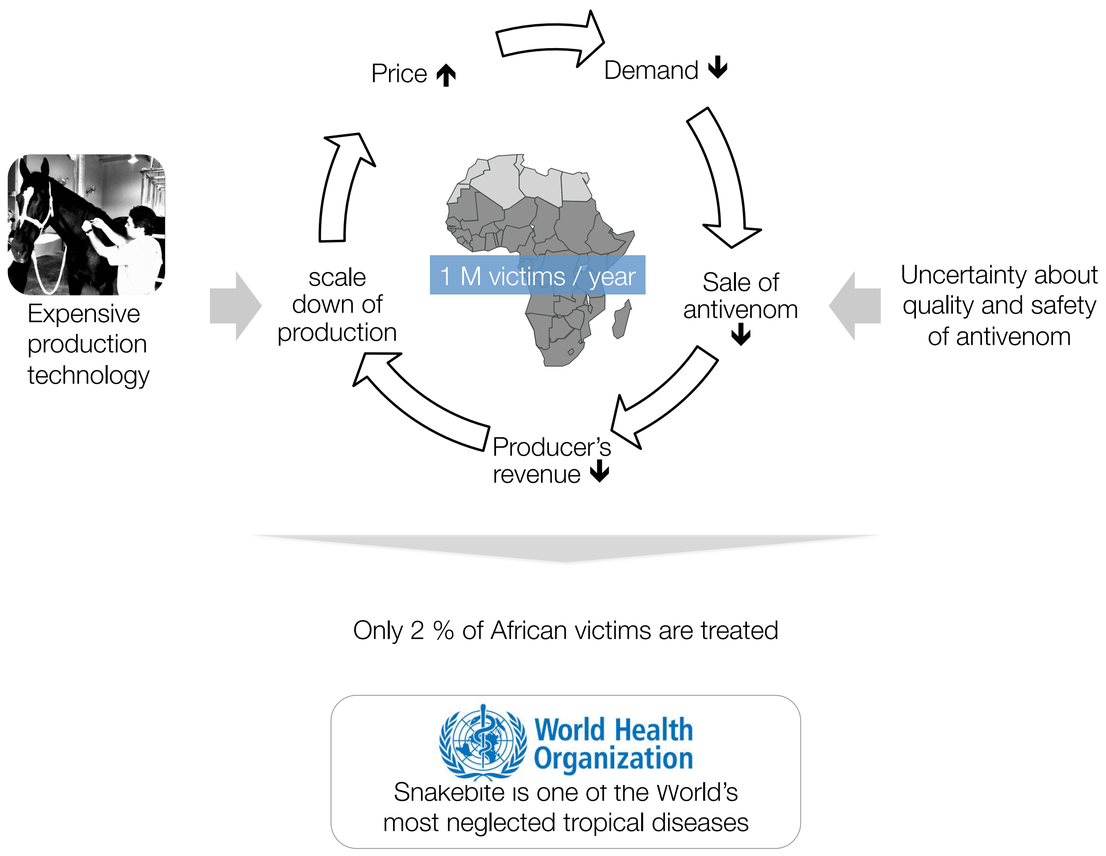


Figure 1 – The Sub-Saharan antivenom crisis. Overview of the vicious cycle fueled by the current production method of antivenom and dangerously inappropriate products of Indian origin marketed by unscrupulous manufacturers dominating the unregulated African market [4]. WHO describes snakebites as one of the Worlds most neglected tropical diseases and the antivenom situation in Sub-Saharan Africa as a long-standing crisis [5].

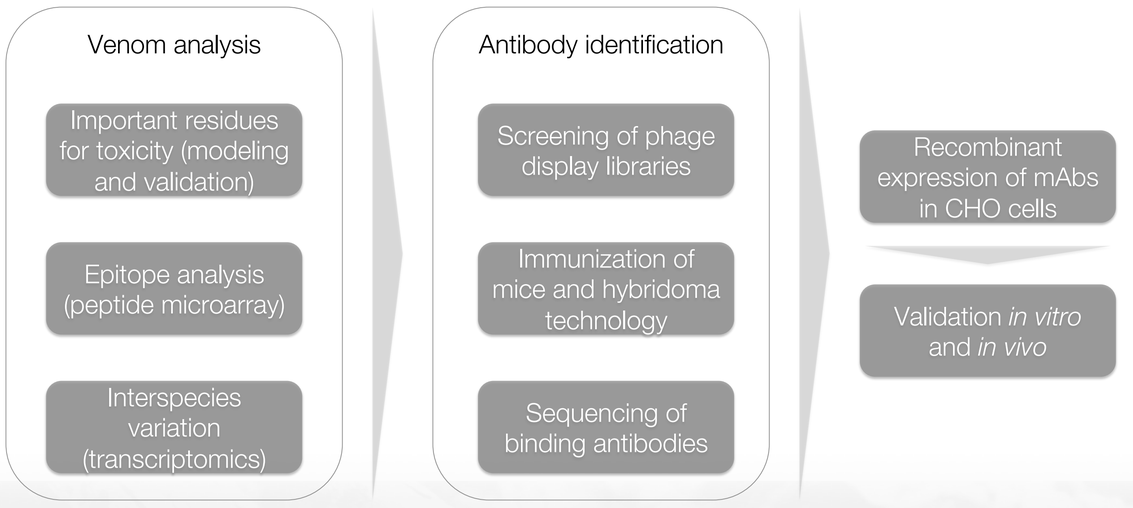


Figure 2 – Schematic overview of research approach.

Modeling Short Neurotoxin 1 from Mamba Snakes

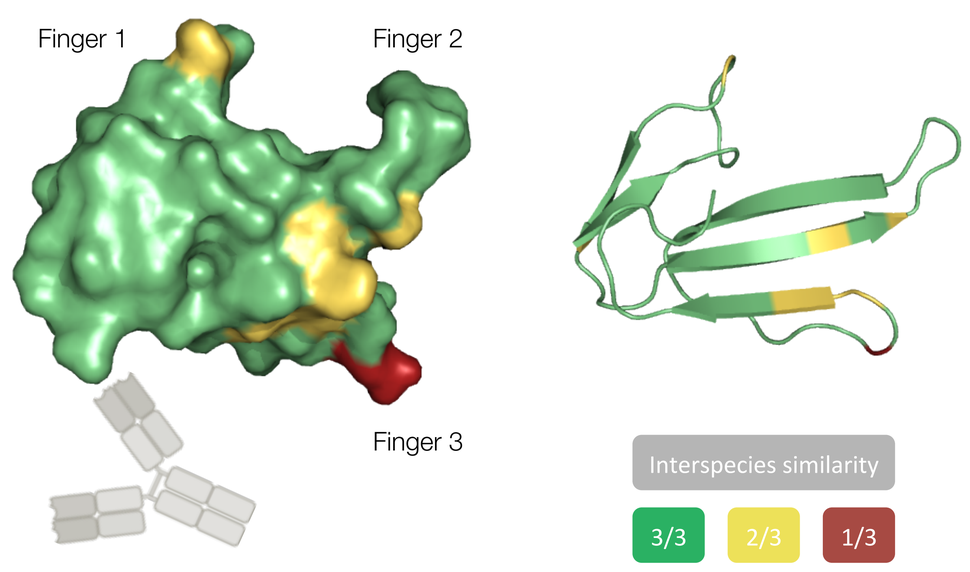
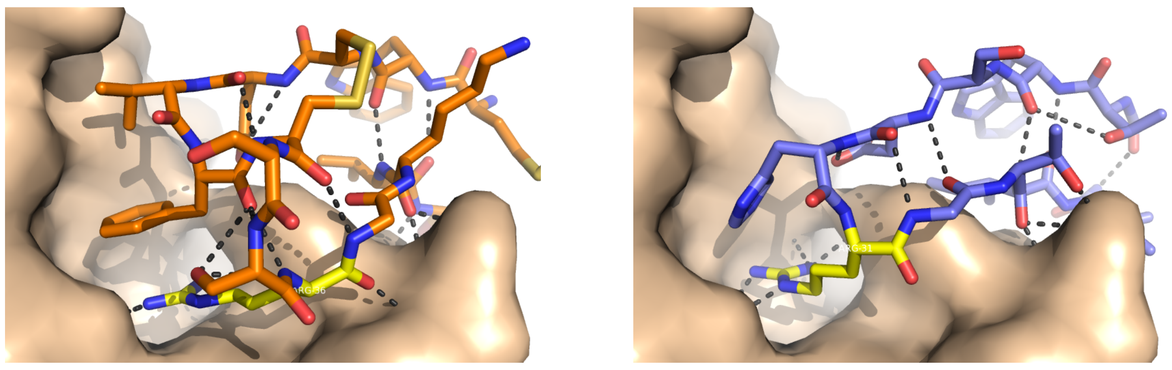


Figure 3 – Homology model of clinically relevant toxin from mamba (*Dendroaspis*) snakes. Surface and cartoon representations of short neurotoxin 1 (SN1) illustrating interspecies variation and the idea of finding one cross-reactive mAb. SN1 is a member of the large and diverse family of three-fingered toxins (3FTx). SN1 is known to antagonize the neuromuscular nicotinic acetylcholine receptor (nAChR) using finger 1 and 2 for binding [6]. Templates for homology model: 3ERA (crystal structure of mutant related Erabutoxin a from a sea snake with low affinity for nAChR) [7] and 2QC1 (nAChR bound distantly related α-bungarotoxin from the many-banded krait) [8].

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sp|P01417|NXS1_DENJA      RICYNHQSTTPATTKSC--GENSCYKKTWSD----HRGTIIERGCG--CPKVKQGIHLHCCQSDKCNN------- 60
sp|P01418|NXS1_DENVI      RICYNHQSTTPATTKSC--GENSCYKKTWSD----HRGTIIERGCG--CPKVKRGVHLHCCQSDKCNN------- 60
sp|P01416|NXS1_DENPO      RICYNHQSTTRATTKSC--EENSCYKKYWRD----HRGTIIERGCG--CPKVKPGVGIHCCQSDKCNY------- 60
sp|P60616|NXL1V_BUNMU     -IVCHTTATSPISAVTCPPGENLCYRKMWCDVFCSSRGKVVELGCAATCPSKKPYEEVTCCSTDKCNPHPKQRPG 74
                           *  :  :*:  :: :*   ** **:* * *     **.::* **.  **. *    : **.:****
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Figure 4 – Identification of residues important for toxicity using homology modeling. Left: Key interactions between nAChR and finger 2 of the co-crystalized α-bungarotoxin (orange). R36 highlighted (yellow). **Right:** Homology model of mamba SN1 aligned to nAChR reveals an arginine residue, R31 (yellow), at a similar position in binding pocket. **Below:** Alignment of the three mamba SN1s and α-bungarotoxin.

Challenges in the near future

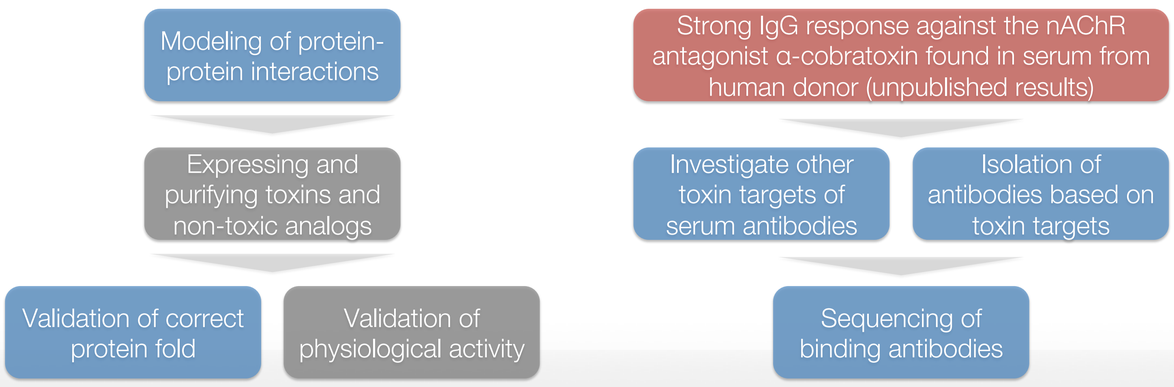

Figure 5 – Schematic overview of upcoming challenges related to protein research.

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