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Epitopic Profiling of Antibody Response against Neurotoxins from the Black Mamba (*Dendroaspis polylepis*)

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1. Introduction

The black mamba (*Dendroaspis Polylepis*) is among the most dangerous snakes in the world, with a venom dominated by three-finger toxins and dendrotoxins¹. Among the three-finger toxins, the α -neurotoxins (α -NT) are the most important², and these are conserved between snake species.

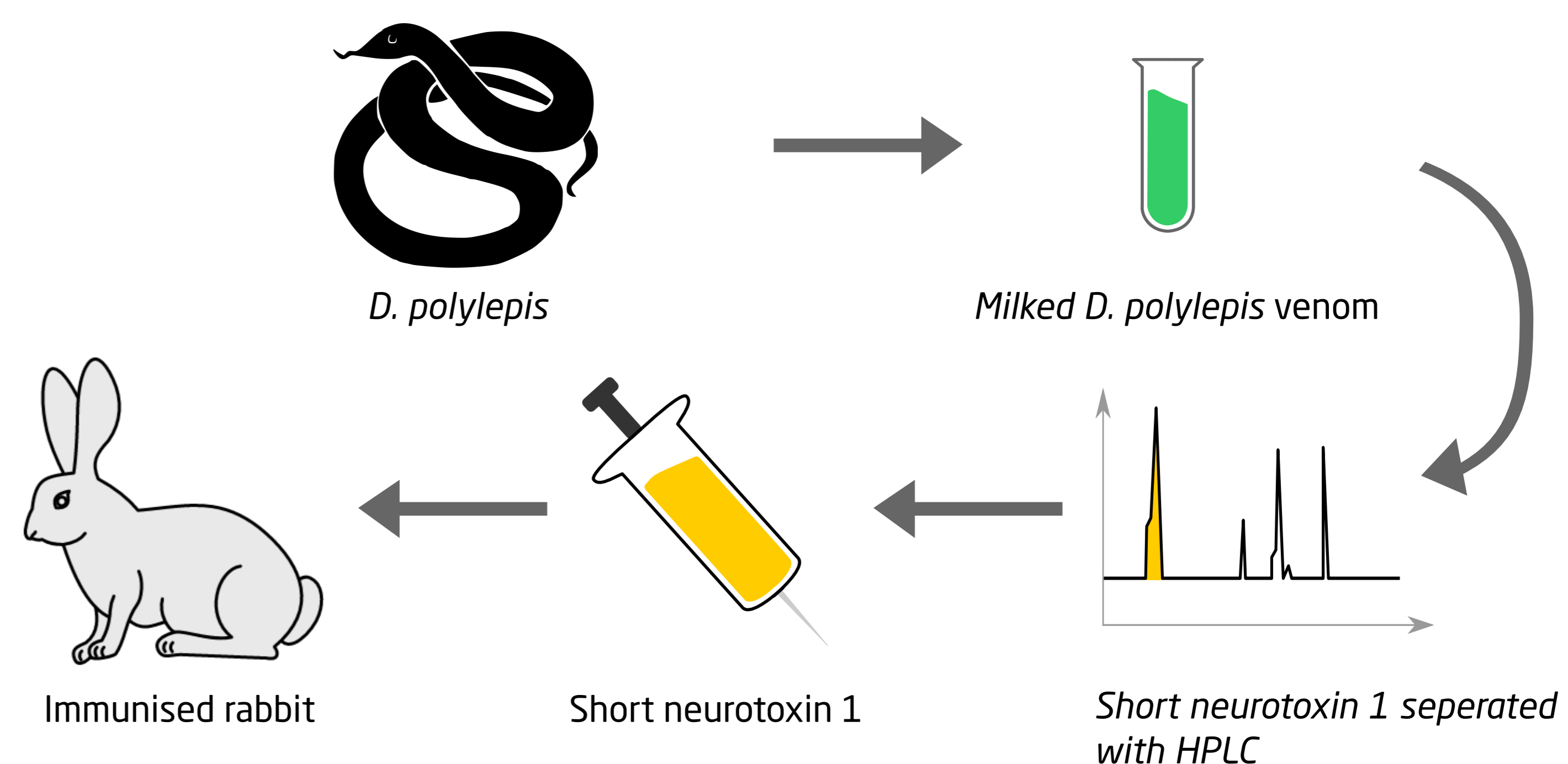
Cross-reactivity between three-finger toxins is known to occur, and understanding this phenomenon in depth may help guide future design of antivenoms to obtain optimal specificity against medically important toxins from different snake species.

Using a bioinformatic approach, we investigated the cross-reactivity between three-finger toxins for a rabbit antiserum raised against short neurotoxin 1 from *D. polylepis* (SN1-DP).



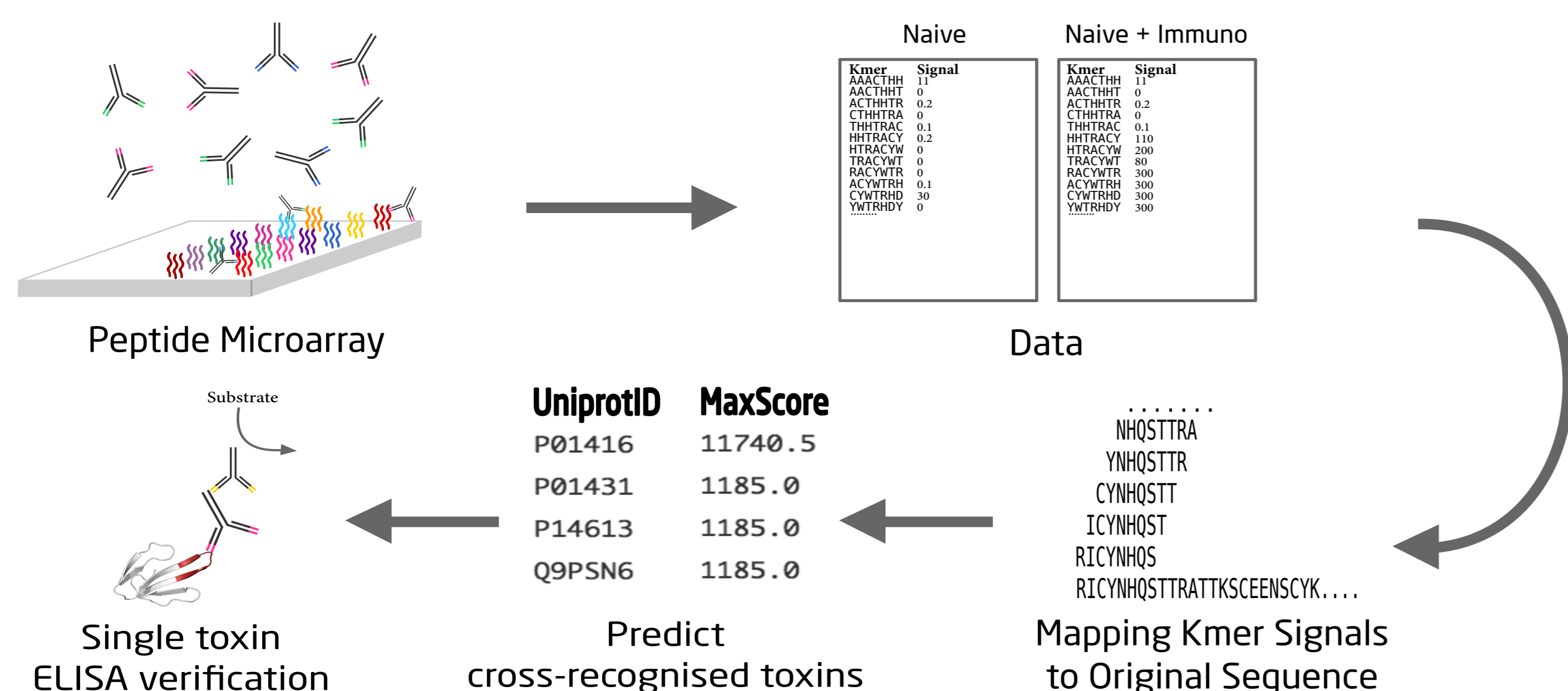
2. Immunisation

The venom of *D. polylepis* was obtained, SN1-DP was isolated and used for immunisation of a rabbit.



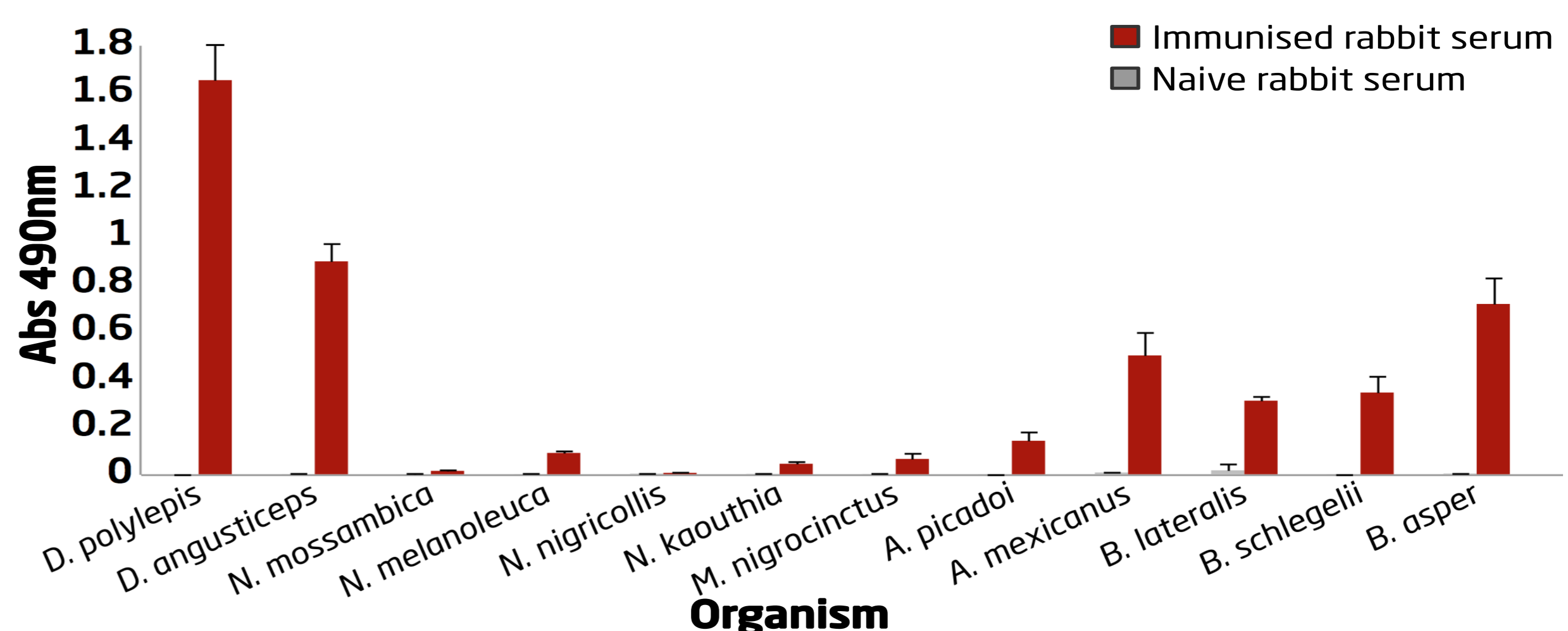
3. Project Flow

A peptide microarray covering all possible epitopes of all known three-finger toxins was synthesised, and binding of antibodies from the rabbit serum to individual toxin epitopes was detected and mapped back to toxin structures.



4. ELISA

An ELISA experiment containing various snake venoms was performed using the rabbit antisera immunised with SN1-DP. This shows reactivity with *D. polylepis* whole venom, strong cross-reactivity with the related *D. angusticeps* venom, and some cross-reactivity with very unrelated venoms.



5. Mapped Signals

A sequence markup plot was constructed for all toxins within the same sub-subfamily of positive control SN1-DP (UniprotID: P01416).

Comparing the high antibody binding regions of SN1-DP and the second highest scoring toxin it shows that Y28R possibly has a high impact, which also confirms that Tyrosine (Y) is more common in epitopes than Arginine (R)³. But it does look like it maintains its signal downstream even with the mutations TII35:37YRT. Due to the normalisation of each sequence, it should be noted that all marked positions in P01416 have a larger signal than any signals in the other toxins.

$$AA_{signal} = \sum_{k \in \{8,12,15\}} AA \in k_{mer} \quad MaxScore = \max(AA_{signals})$$

UniprotID	Organism	MaxScore	Sequence
P01416	<i>D. polylepis</i>	11740.5	RICYNHQSSTTRATTKSC--EENSCKYKRYWRDHRGTIERGCGCPKVKPGVGIHCCQSDKCN
P01431	<i>N. mossambica</i>	1185.0	LECHNQSSSEPTTTRCSGGETNCKYKRWDRHGRYRERGGCPVSKNGIEINCCCTDRCNN
P14613	<i>N. kaouthia</i>	1185.0	LECHNQSSIQTPTTTGCSGGETNCKYKRWDRHGRYRERGGCPVSKNGIEINCCCTDRCNN
Q9PSN6	<i>N. sputatrix</i>	1185.0	LECHNQSSSQPTTTGCSGGETNCKYKRWDRHGRYRERGGCPVSKNGIEINCCCTDRCNN
P60770	<i>N. atra</i>	1185.0	LECHNQSSSQPTTTGCSGGETNCKYKRWDRHGRYRERGGCPVSKNGIEINCCCTDRCNN
P60771	<i>N. kaouthia</i>	1185.0	LECHNQSSSQPTTTGCSGGETNCKYKRWDRHGRYRERGGCPVSKNGIEINCCCTDRCNN
Q9PTT0	<i>N. naja</i>	1185.0	LECHNQSSSQPTTTGCSGGETNCKYKRWDRHGRYRERGGCPVSKNGIEINCCCTDRCNN
Q9Y616	<i>N. sputatrix</i>	1110.0	LECHNQSSSEPTTTRCSGGETNCKYKRWDRHGRYRERGGCPVSKNGIEINCCCTDRCNN
Q9Y615	<i>N. sputatrix</i>	1110.0	LECHNQSSSQPTTTGCSGGETNCKYKRWDRHGRYRERGGCPVSKNGIEINCCCTDRCNN

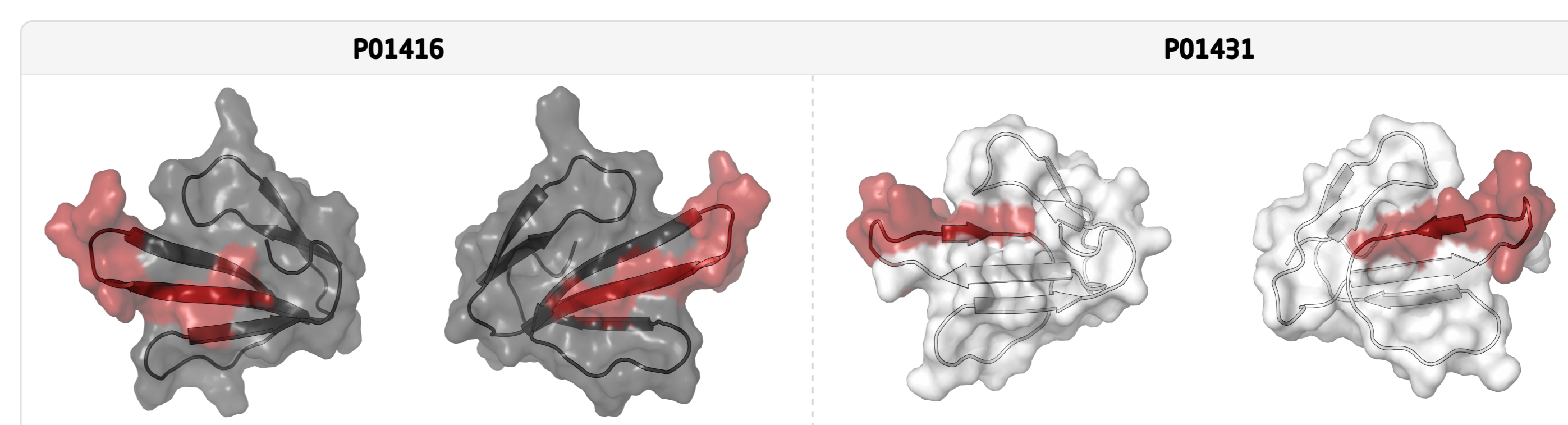
6. Structural Comparison

Due to the observed elimination of the start region on the sequence markup plot, the top toxin (P01431) was modelled due to the lack of a PDB structure using SWISS-MODEL⁴.

The structure for SN1-DP (PDB: 1NTX) and the modelled P01431 structure were compared in PyMOL⁵, and their amino acid sequences showing a high degree of antibody binding were marked in red.

Red regions:

P01416: RICYNHQSSTTRATTKSC--EENSCKYKRYWRDHRGTIERGCGCPKVKPGVGIHCCQSDKCN
 P01431: LECHNQSSSEPTTTRCSGGETNCKYKRWDRHGRYRERGGCPVSKNGIEINCCCTDRCNN



Conclusion & Future

A high chance of antivenom cross-reactivity between short neurotoxins from different snake species exists due to conservation of important epitopes in these toxins. Future ELISA experiments containing SN1-DP and some of the toxins with high signal will be conducted to further support the results observed in this bioinformatic analysis.

References

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- PyMOL Version 1.7.4 Schrödinger, LLC
- Black Mamba image, Herman Pijpers - www.flickr.com (Adapted)

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