Development of an innovative magnetic-beads based methodology to enhance antivenomics capabilities.

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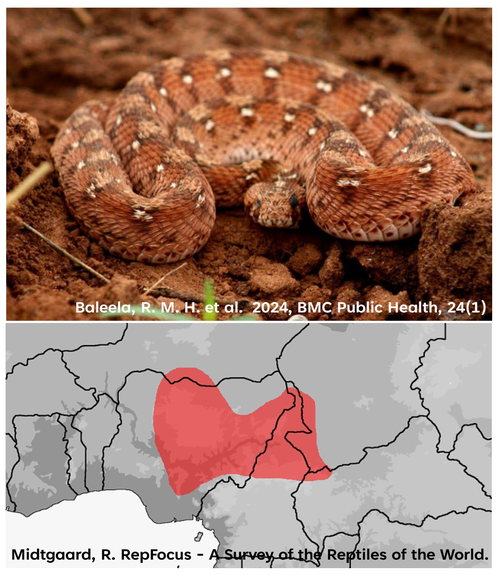
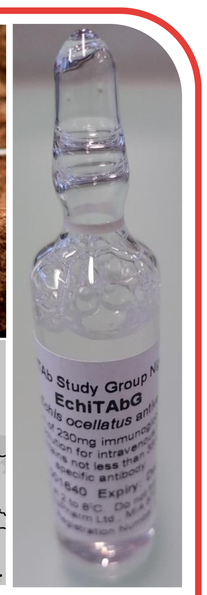
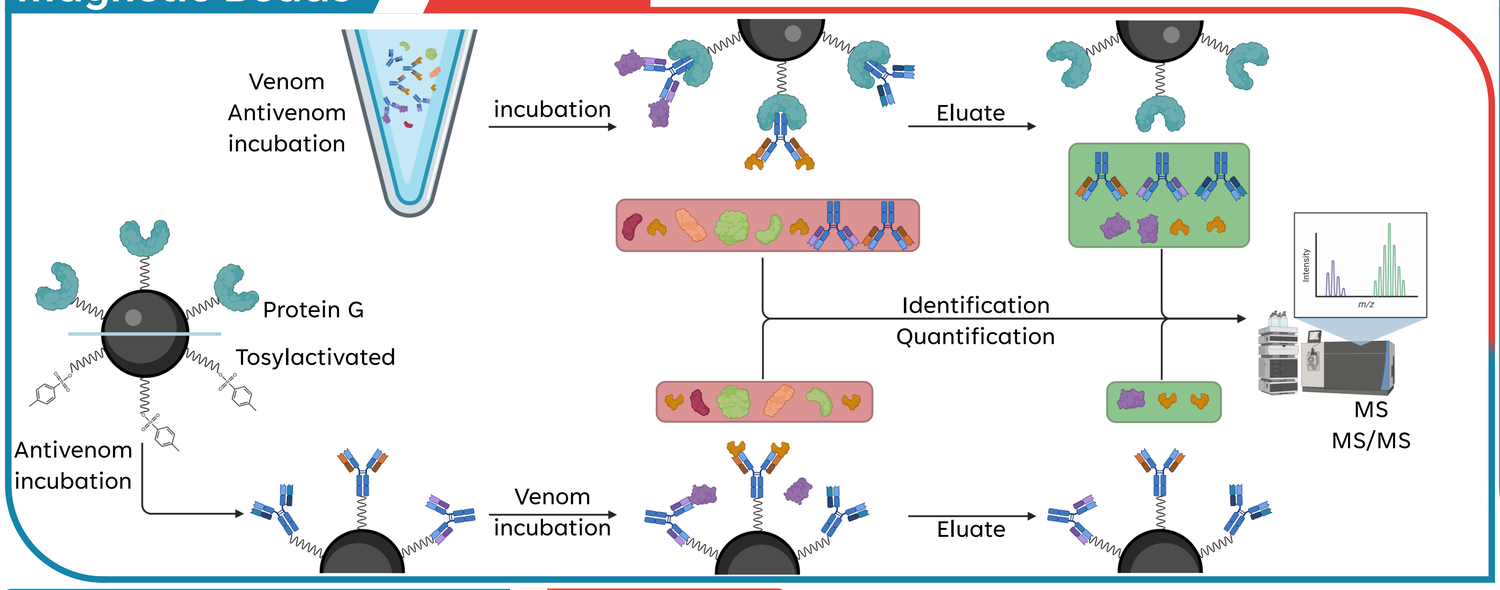
Introduction

- In September 2023, WHO estimated 1.8–2.7 million snake envenomation cases annually, causing 80k–138k deaths.[1]
- Antivenoms, composed of venom immunized animal IgGs, are the only available treatment but have limitations.
- Research is exploring alternatives like ADDomers[2], nanobodies, and monoclonal antibodies[3].
- Assessing antivenom efficacy in vitro through Antivenomics remains essential.
- No major advances in Antivenomics have been made since 2017.
- We are developing a novel technique using magnetic beads to reduce the consumption of antivenoms and venoms. In addition, Biolayer Interferometry is used to define the apparent dissociation constant (K_D^*)
- This methodology uses *Echis romani* venoms against EchiTabG, a monospecific whole IgG antivenom for *Echis ocelatus* that shows cross-reactivity with *Echis coloratus* venom.

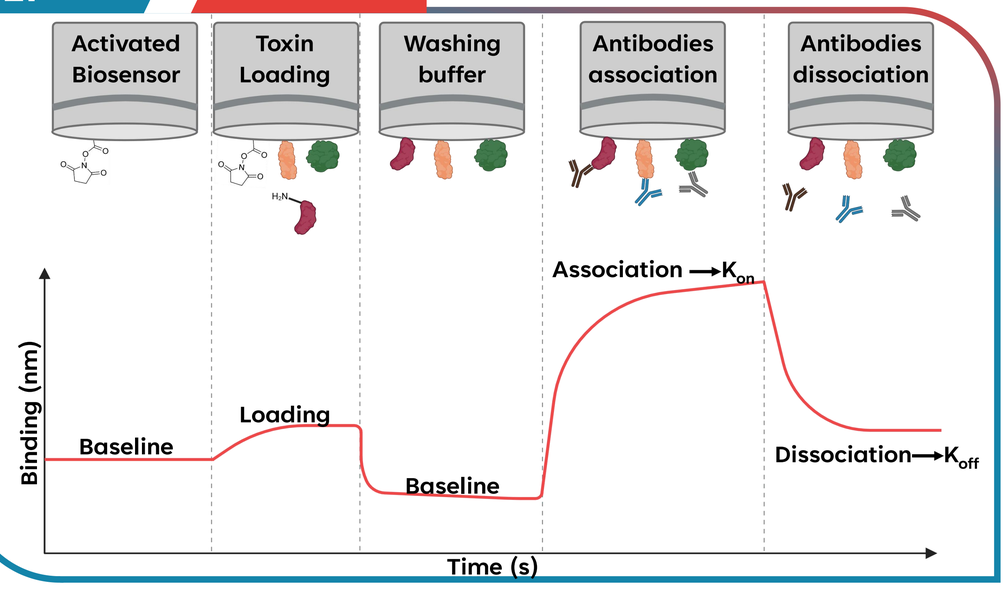




Magnetic Beads

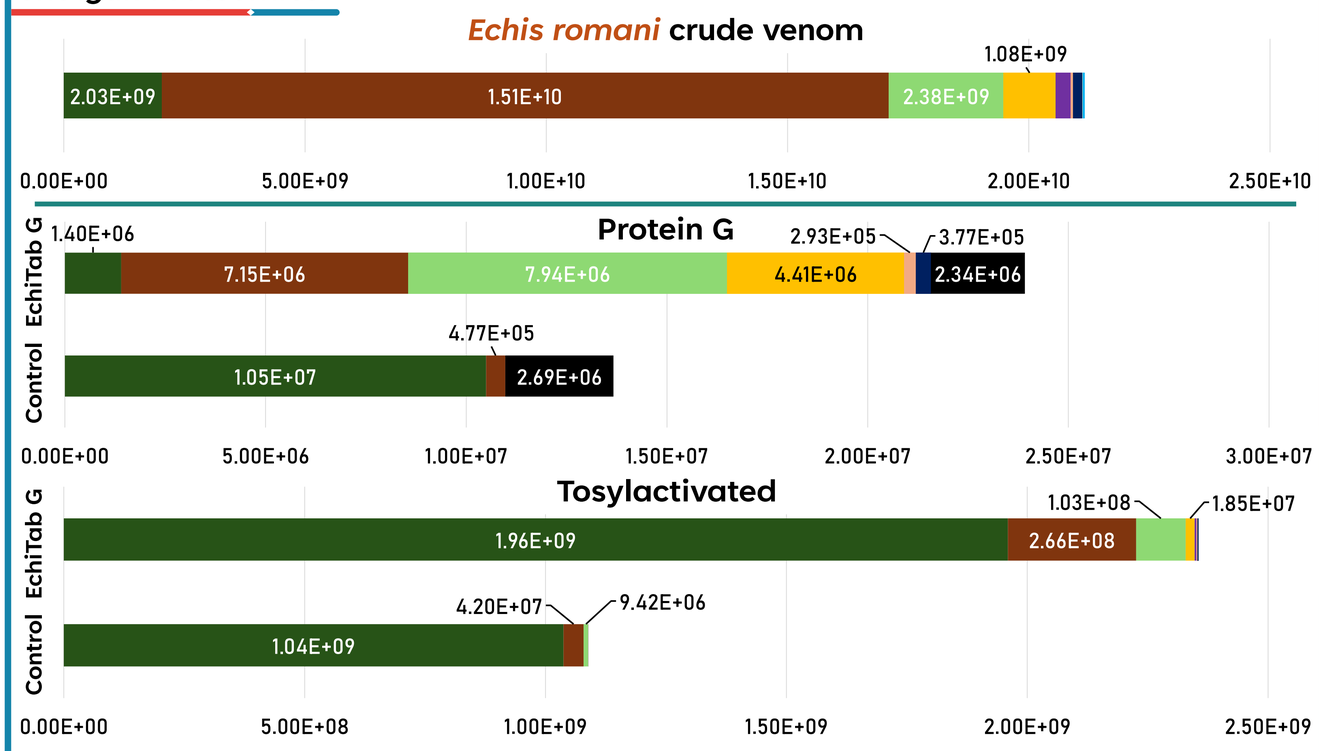


BLI



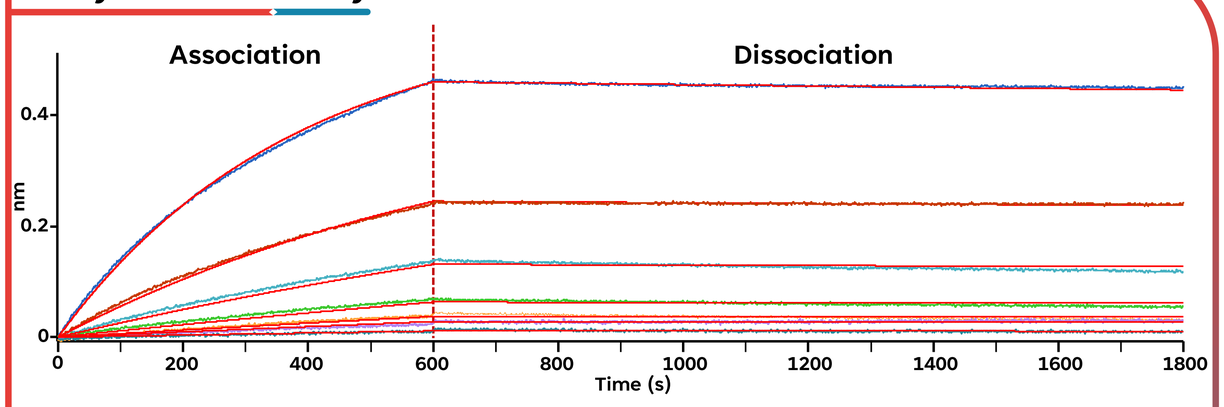
Results & Discussion

Shotgun Proteomics



- Relative quantification was performed using the three most ions from the tryptic digestion.
- A control experiment with naïve sheep IgG was included.
- Significant differences were observed between Protein G and Tosylactivated beads, likely due to differences in IgG orientation.
- A higher toxin diversity was captured using Protein G magnetic beads.
- The control suggests that a substantial proportion of the detected PLA_2s are involved in non-specific interactions.
- In contrast, a significant fraction of PLA_2s is also captured specifically.
- In addition, SVMPs appear to be captured almost exclusively through specific interactions

Biolayer Interferometry



K_D^* (M)	K_D^* error	k_{on}^* (1/M.s)	k_{on}^* Error	k_{off}^* (1/s)	k_{off} error	R^2
$5.11*10^{-10}$	$4.24*10^{-12}$	$5.29*10^4$	68.1	$2.70*10^{-5}$	$2.22*10^{-7}$	0.9993

- The K_D^* was determined using a 1:1 binding model.
- Although this model typically describes the interaction between one binder and one ligand, it fits our system particularly well.
- Therefore, the 1:1 model was used to describe the apparent dissociation constant between one antivenom (EchiTab G) and one venom (*Echis romani*).
- The apparent dissociation constant ($K_D^* = 5.11 \times 10^{-10}$ M) is consistent with affinities typically observed following animal hyperimmunisation.

Next step

LC fractionation | BLI measurement | LC-MS/MS identification

Conclusions

- This methodology provides results consistent with current knowledge, with quantification matching previously published studies.
- While the method may seem non-specific, SVMPs were specifically captured, suggesting stricter washing could improve specificity. Non-specific interactions appear limited to PLA2s, likely due to intrinsic properties of this toxin family.
- The combination of Antivenomics and BLI offer deeper insight into Venom-Antivenom Interactions particularly in terms of affinity, diversity and recognition.
- In addition, these two methodologies allow the use of less medicaly relevant compounds and could be applied in routine quality control.

Refs & Acks

[1] WHO. Snakebite envenoming. *14 January 2025* https://www.who.int/news-room/fact-sheets/detail/snakebite-envenoming (2023)

[2] Menzies, S. K et *al.* (2023). ADDovenom: Thermostable Protein-Based ADDomer Nanoparticles as New Therapeutics for Snakebite Envenoming. *Toxins*, *15*(12).

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