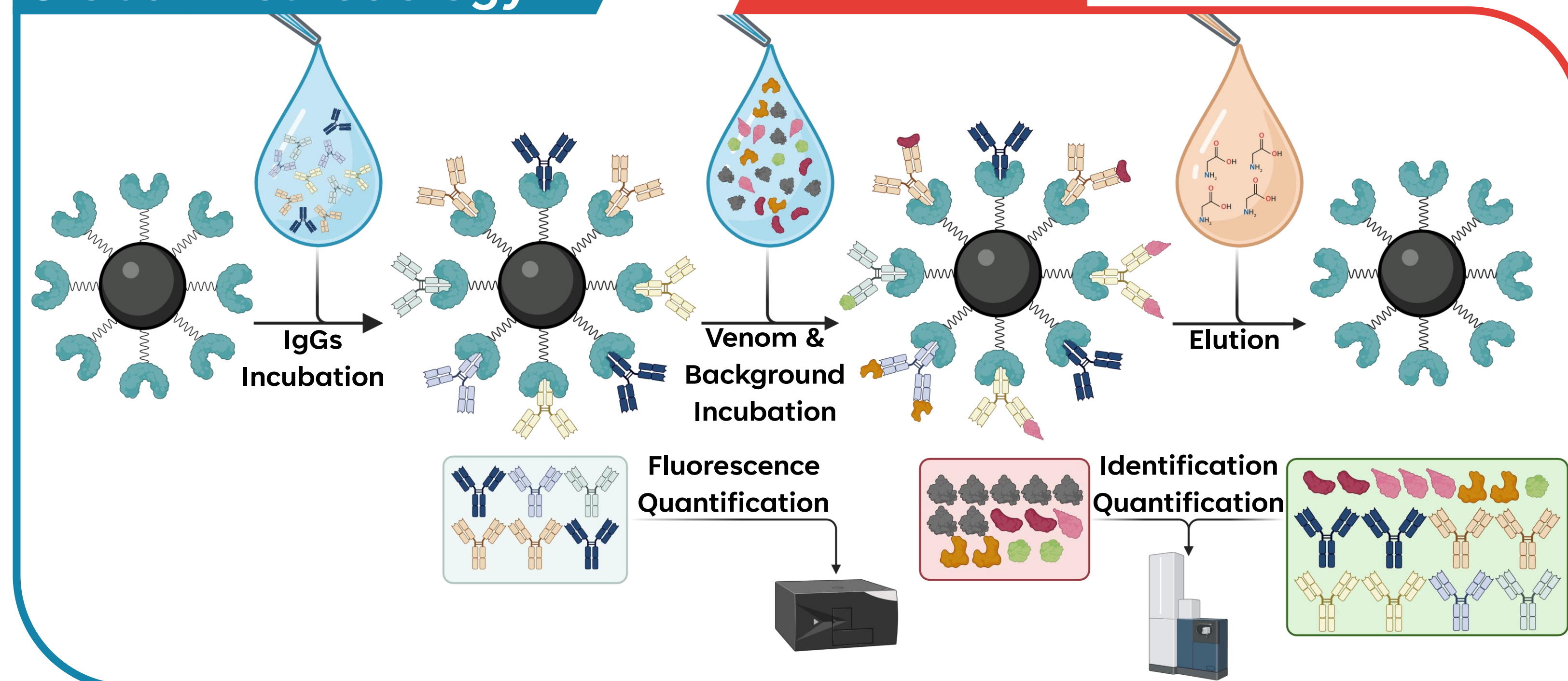


Introduction

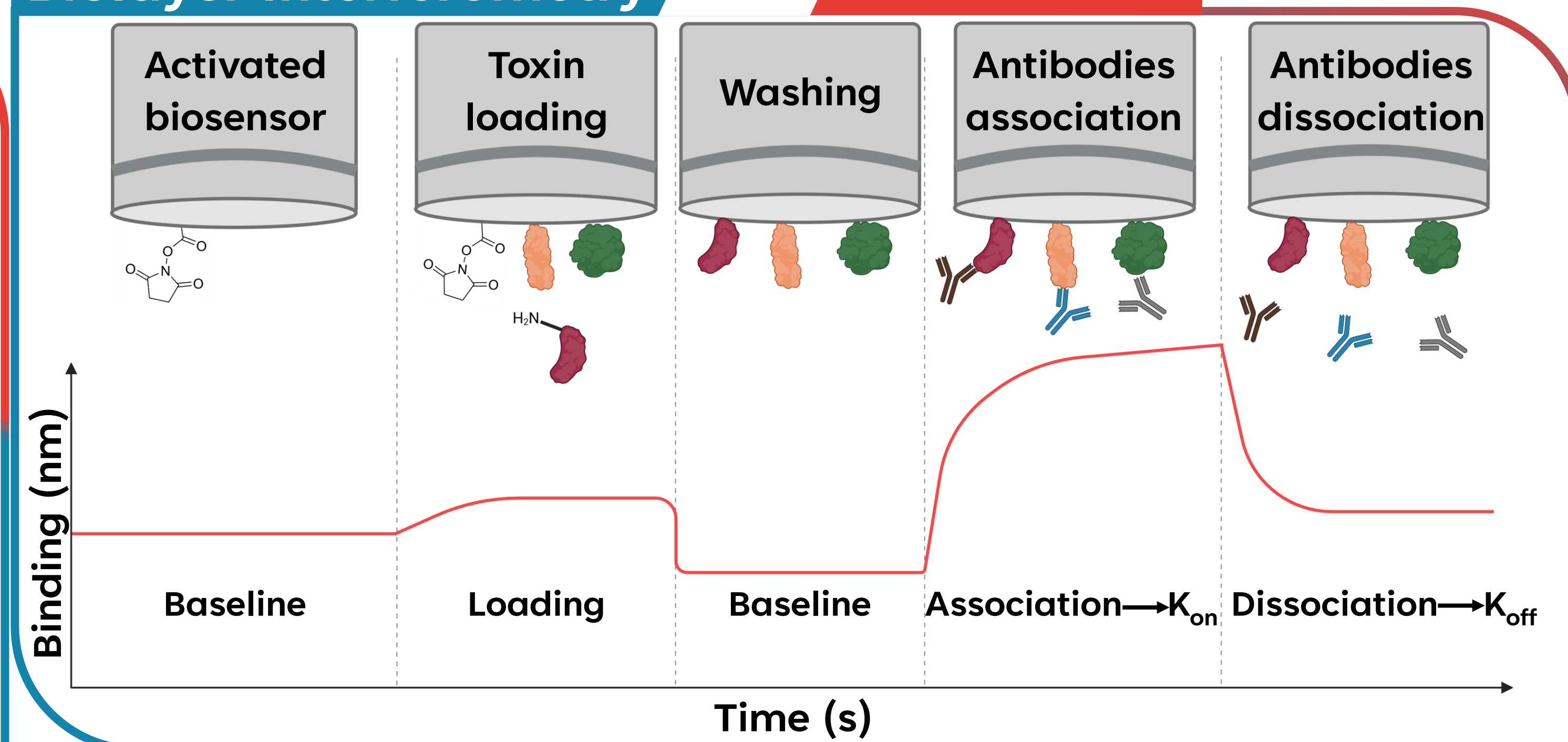
- In 2023, WHO estimated 1.8–2.7 million snake envenomation cases annually, causing 80k–138k deaths.¹
- Antivenoms, composed of venom immunized animal IgGs, are the only available treatments but face limitations.
- Research is exploring innovative alternatives like ADDomers², nanobodies, and monoclonal antibodies³.
- Assessing antivenom efficacy in vitro through antivenomics remains essential, but no major advances in antivenomics have been made since 2017.
- In that context, this work presents a novel approach to assessing antivenom efficacy using magnetic beads to reduce the consumption of antivenoms and venoms, while keeping all the needed information. BioLayer Interferometry is additionally used to define the apparent dissociation constant (K_D^*)
- The proof-of-concept is demonstrated with *Echis romani* venom against EchiTABG, a monospecific antivenom prepared for this species, and Protein G magnetic beads.
- Protein G beads form non-covalent, Fc-specific interactions, ensuring better IgG orientation.



Global methodology

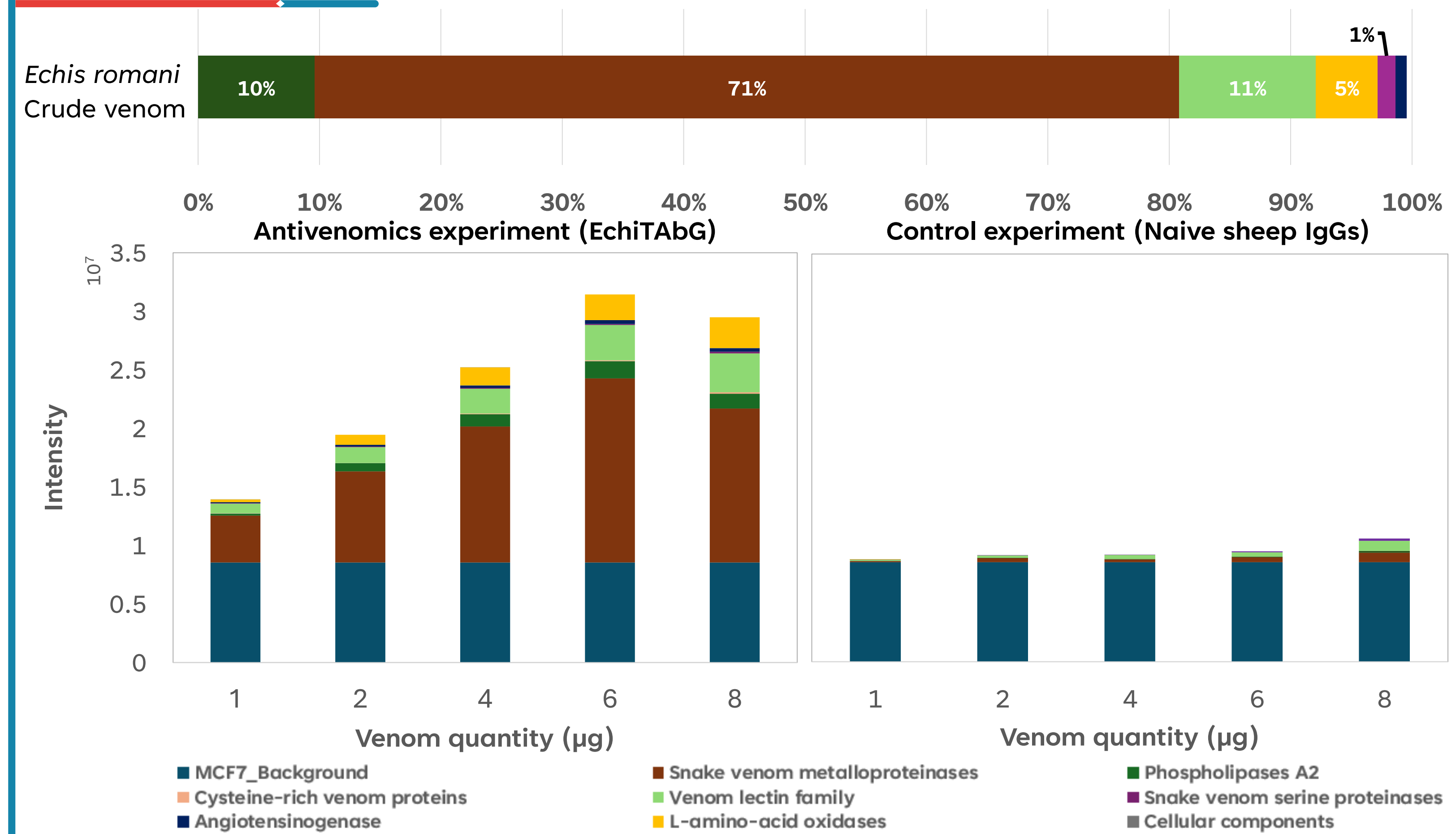


BioLayer Interferometry



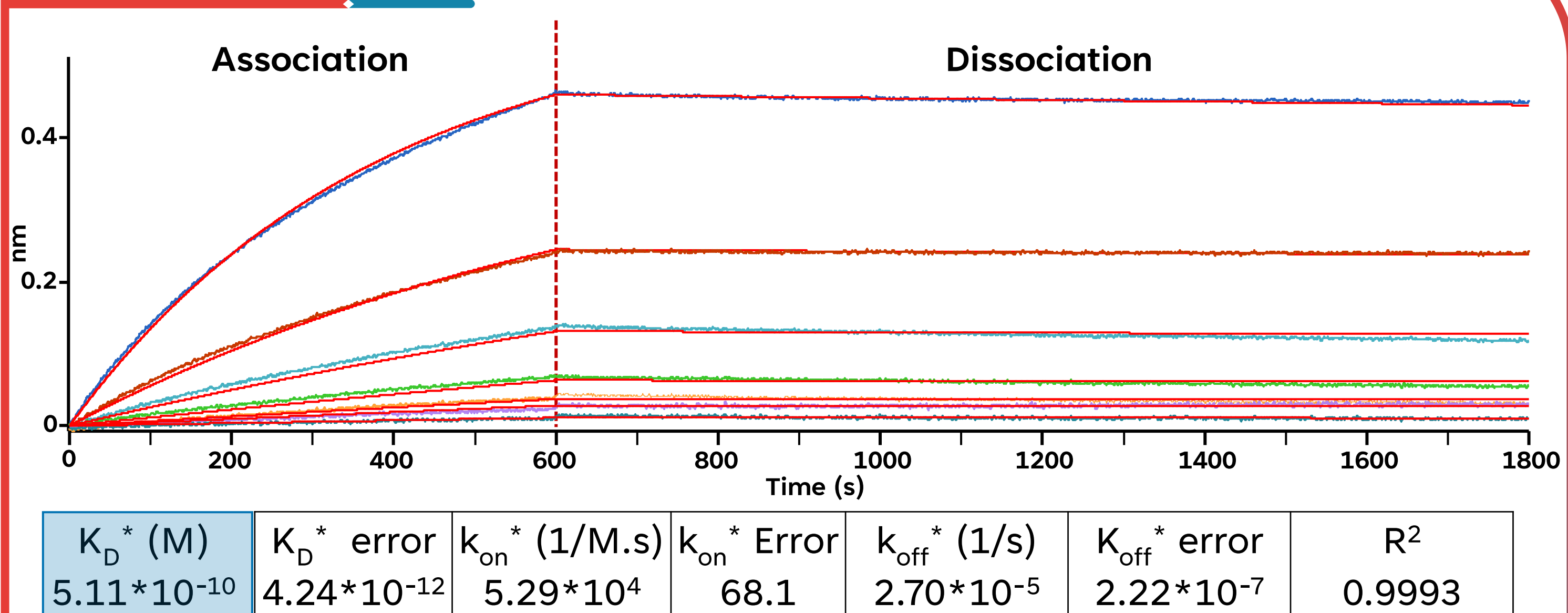
Results & Discussion

Shotgun Proteomics



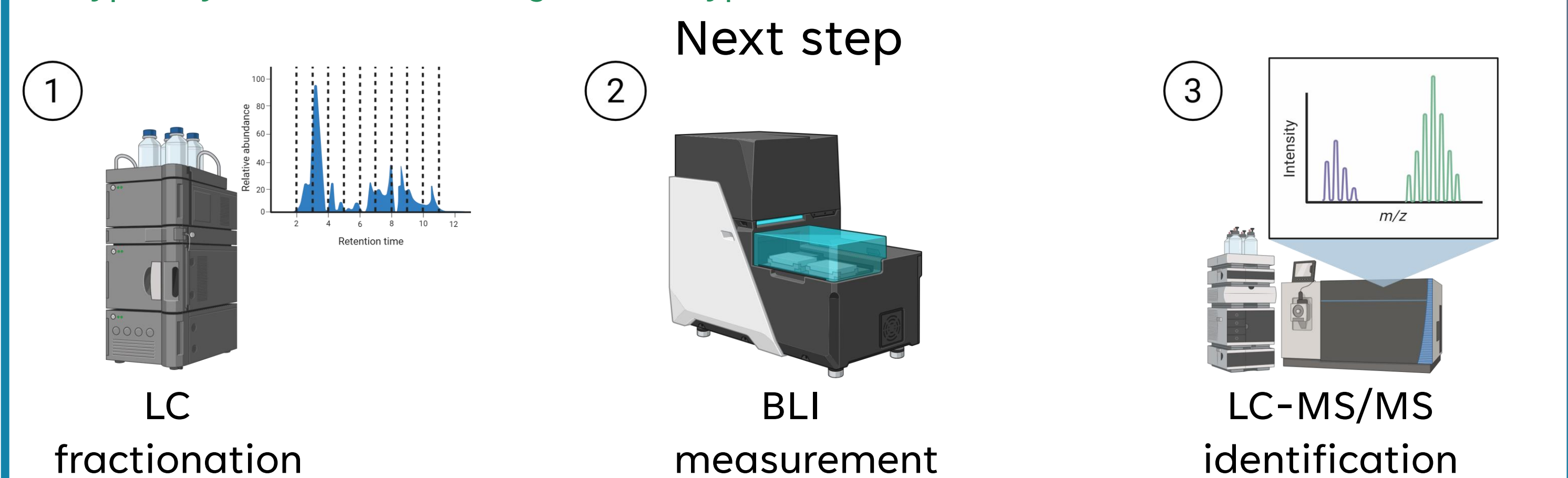
- Label-free quantification was performed using MS1 intensity-based measurements.
- Intensity normalization was carried out using the global median of background protein intensities.
- Toxin intensities increased proportionally to the amount of toxin incubated, reaching a maximum before declining once saturation occurred.
- In contrast, during the control experiment, toxin signals remained a minor component of the total intensity and showed no consistent trend.
- This clear divergence in behavior demonstrates the specificity and robustness of the methodology.
- At saturation, the most efficiently captured toxins were SVMPs, followed by C-type lectins, L-amino acid oxidases, and finally Phospholipases A₂.

BioLayer Interferometry



$$K_D^* = \frac{k_{off}^*}{k_{on}^*}$$

- Apparent 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 (EchiTABG) and *Echis romani* venom.
- The apparent dissociation constant ($K_D^* = 5.11 \times 10^{-10}$ M) is consistent with affinities typically observed following animal hyperimmunization.



Conclusions

- This methodology provides results consistent with current knowledge, with quantification matching previously published studies.
- Toxins were specifically captured
- Non-specific interactions appear to be limited in regards of the control experiment.
- The combination of antivenomics and BLI offers deeper insight into Venom-Antivenom Interactions particularly in terms of affinity, diversity and recognition.
- In addition, these two methodologies allow the use of less medically relevant compounds and could be applied in routine quality control.

Refs & Acks

- WHO. Snakebite envenoming. 14 January 2025 <https://www.who.int/news-room/fact-sheets/detail/snakebite-envenoming> (2023)
- Menzies, S. K et al. (2023). ADDovenom: Thermostable Protein-Based ADDomer Nanoparticles as New Therapeutics for Snakebite Envenoming. *Toxins*, 15(12).
- Dias da Silva, W. et al. (2022). Antibodies as Snakebite Antivenoms: Past and Future. In *Toxins* (Vol. 14, Issue 9). MDPI.

