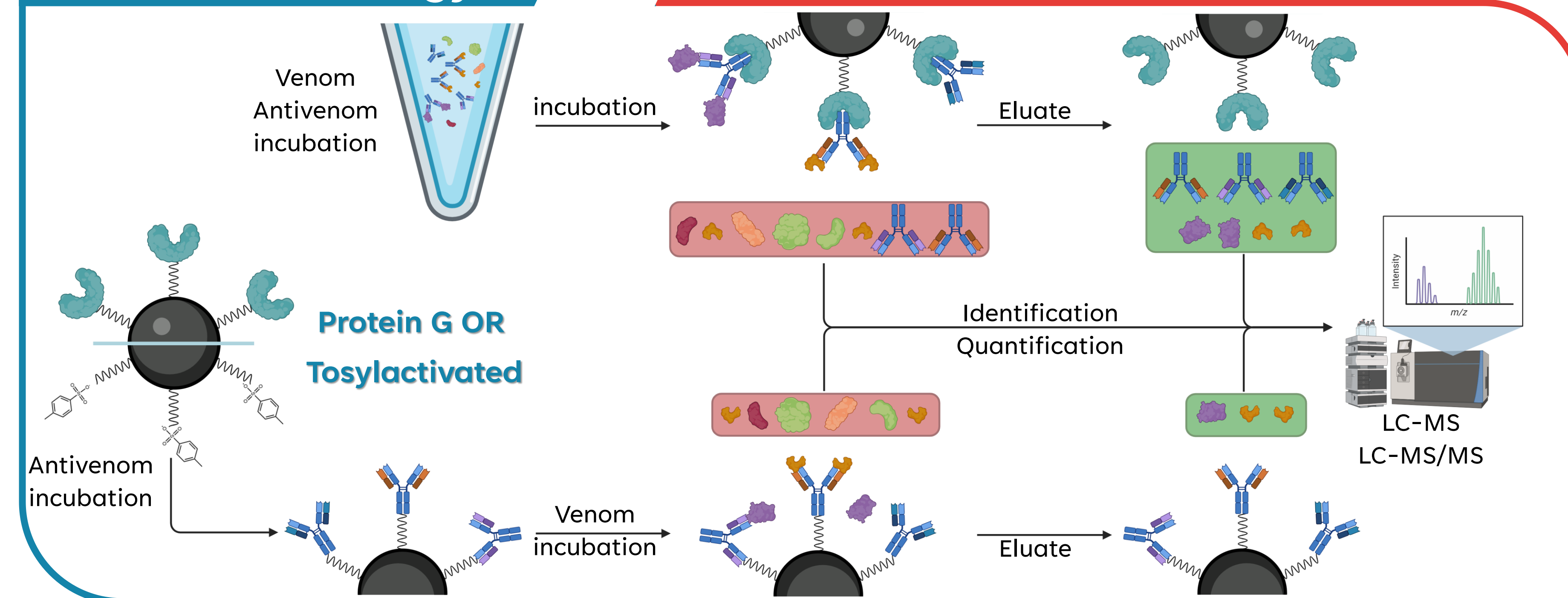


Introduction

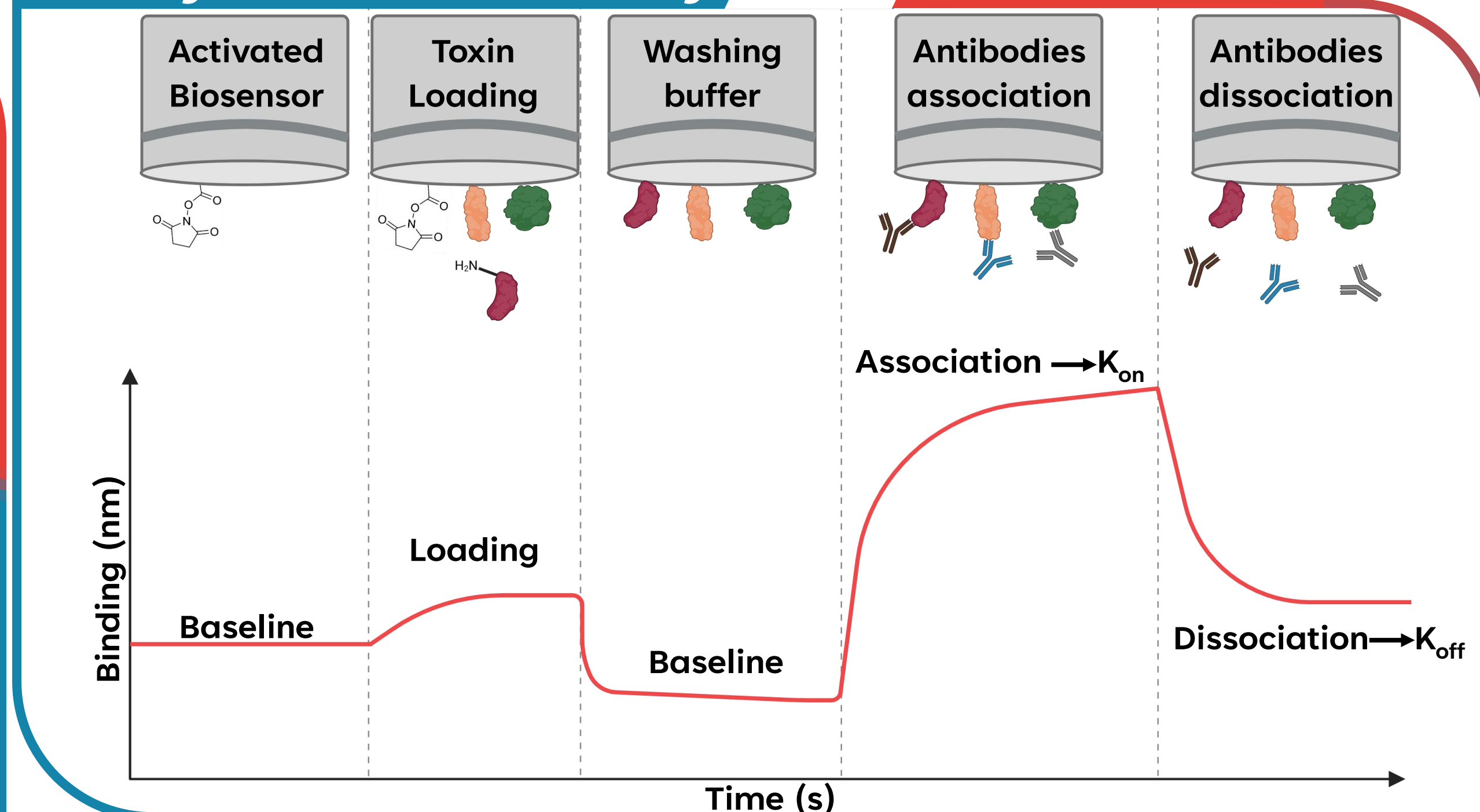
- In 2023, WHO estimated 1.8–2.7 million snake envenomation cases annually, causing 80k–138k deaths.¹
- Antivenoms, composed of venom immunized animal IgGs, are the only available treatments but face limitations.
- Research is exploring innovative alternatives like ADDomers², nanobodies, and monoclonal 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. 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 *Echis ocellatus*, and two chemistries for grafting the antibodies to the beads.
- Protein G beads form non-covalent, Fc-specific interactions, ensuring better IgG orientation and elution, unlike Tosylactivated beads, which bind IgGs covalently and randomly.



Global methodology

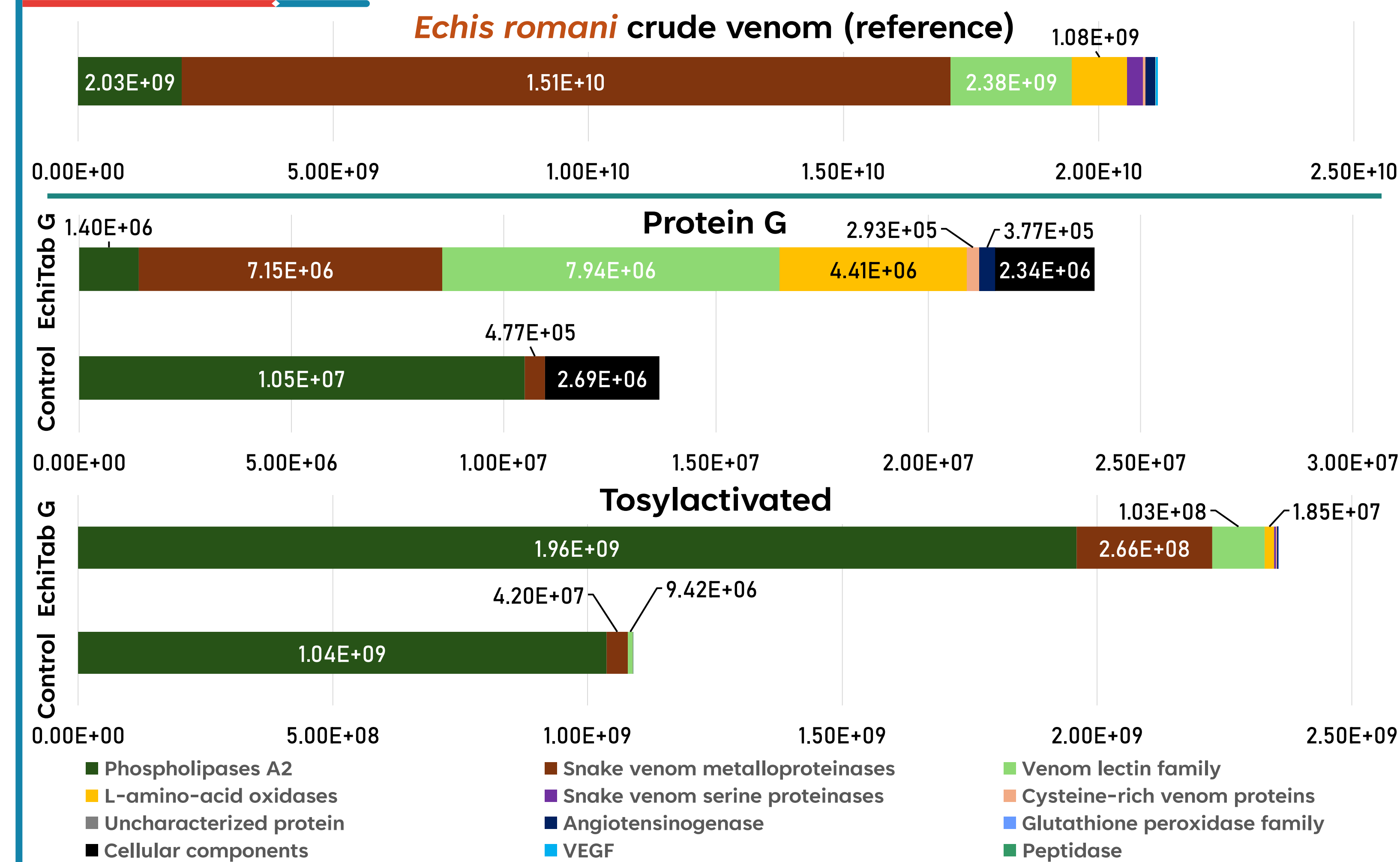


Biolayer Interferometry



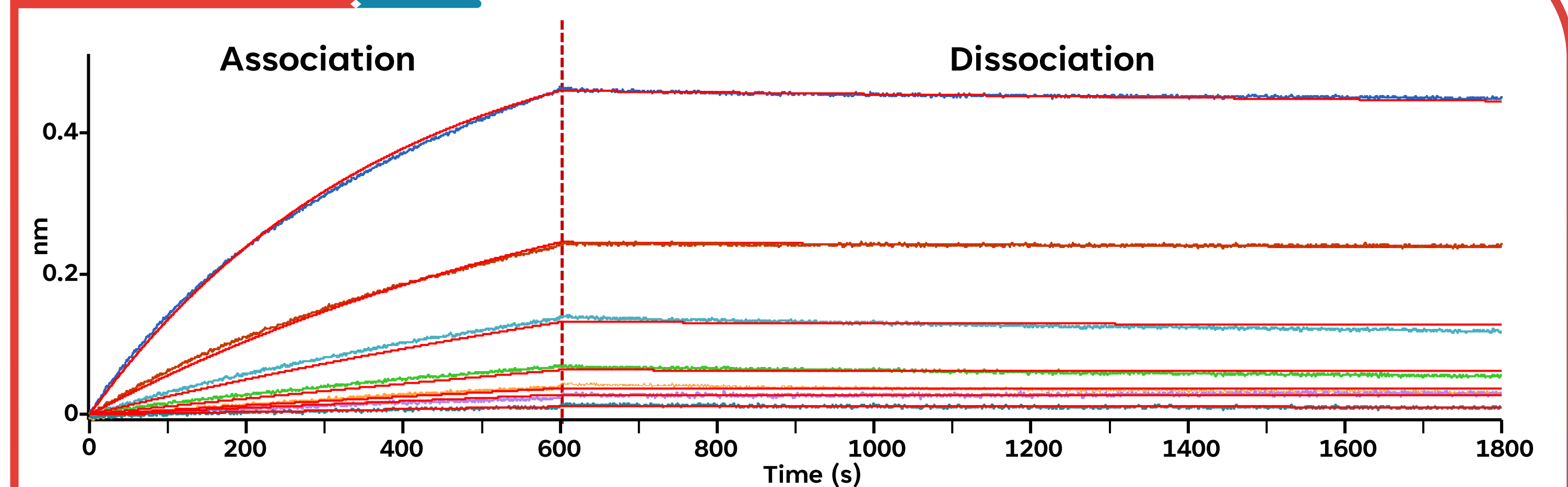
Results & Discussion

Shotgun Proteomics



- Relative quantification of each toxin was performed using the three most intense unique detected ions, from the venom tryptic digestion peptide mixture.
- A control experiment with naïve sheep IgG was included.
- Significant differences were observed between Protein G and tosylactivated beads, likely due to differences in IgGs orientation.
- A higher toxin diversity was captured using Protein G magnetic beads.
- The control suggests that a substantial proportion of the detected PLA₂s are involved in non-specific interactions.
- In contrast, a significant fraction of PLA₂s is also captured specifically.
- In addition, SVMPs appear to be captured almost exclusively through specific interactions.

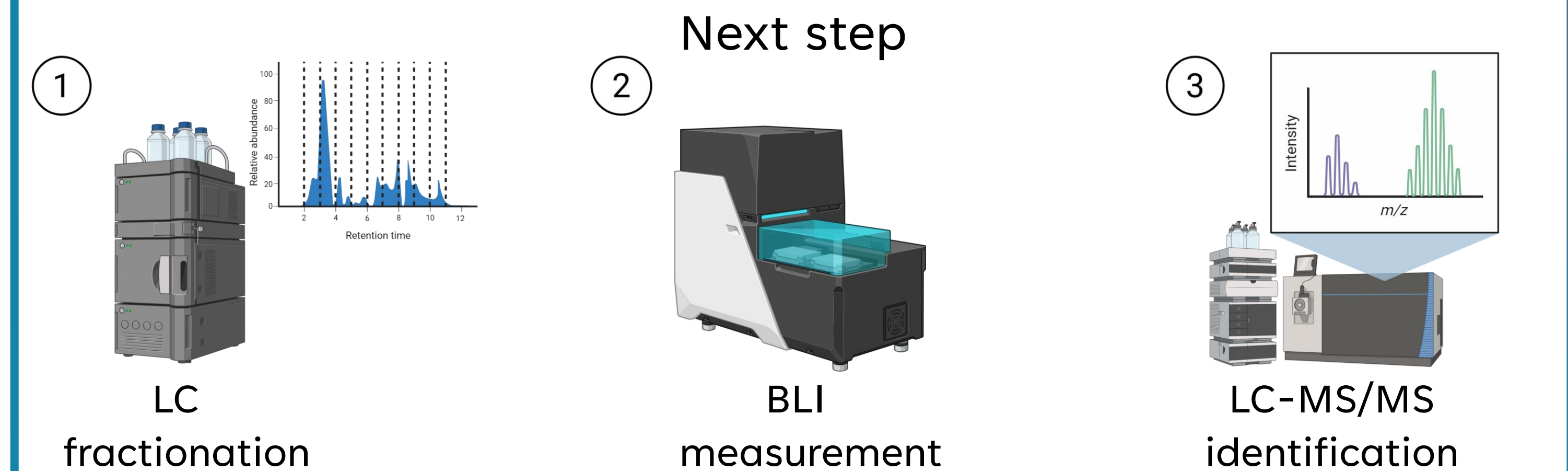
Biolayer Interferometry



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

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

- 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 (EchiTab G) 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.

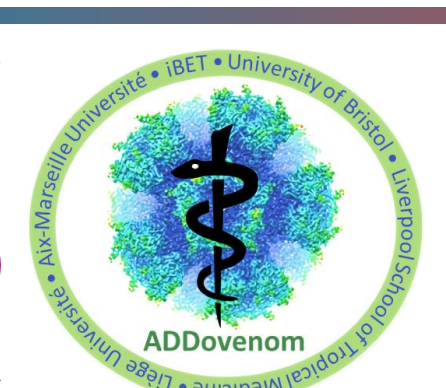


Conclusions

- This methodology provides results consistent with current knowledge, with quantification matching previously published studies.
- SVMPs were specifically captured
- Non-specific interactions appear limited to PLA₂s, likely due to intrinsic properties of this toxin family, suggesting stricter washing could improve specificity.
- 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

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