

Design and Binding Affinity of Antisense Peptides for Snake Venom Neutralization

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Supporting information

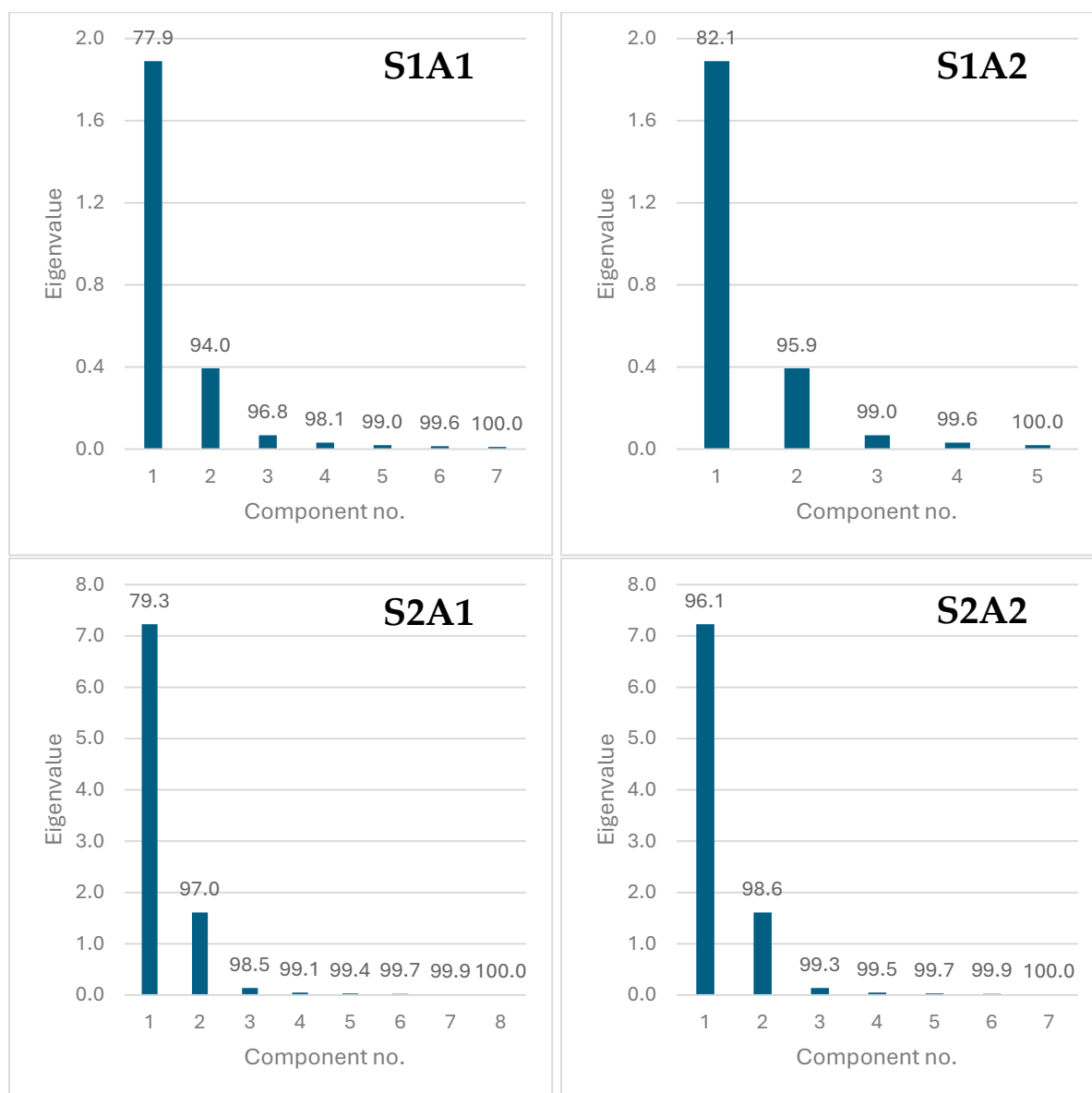


Figure S1. Results of the singular value decomposition (SVD) analysis of fluorescence data for titrations involving antisense peptides S1A1, S1A2, S2A1, and S2A2 (Scree plots; <https://doi.org/10.1198/106186007X256080>). The x-axis represents the component number, and the y-axis shows the square sum of the corresponding singular values. Data labels above each bar display the cumulative percentage of the total variance accounted for by the components. The analysis highlights that the first two components capture the majority of the signal variance, with diminishing contributions from higher-order components.

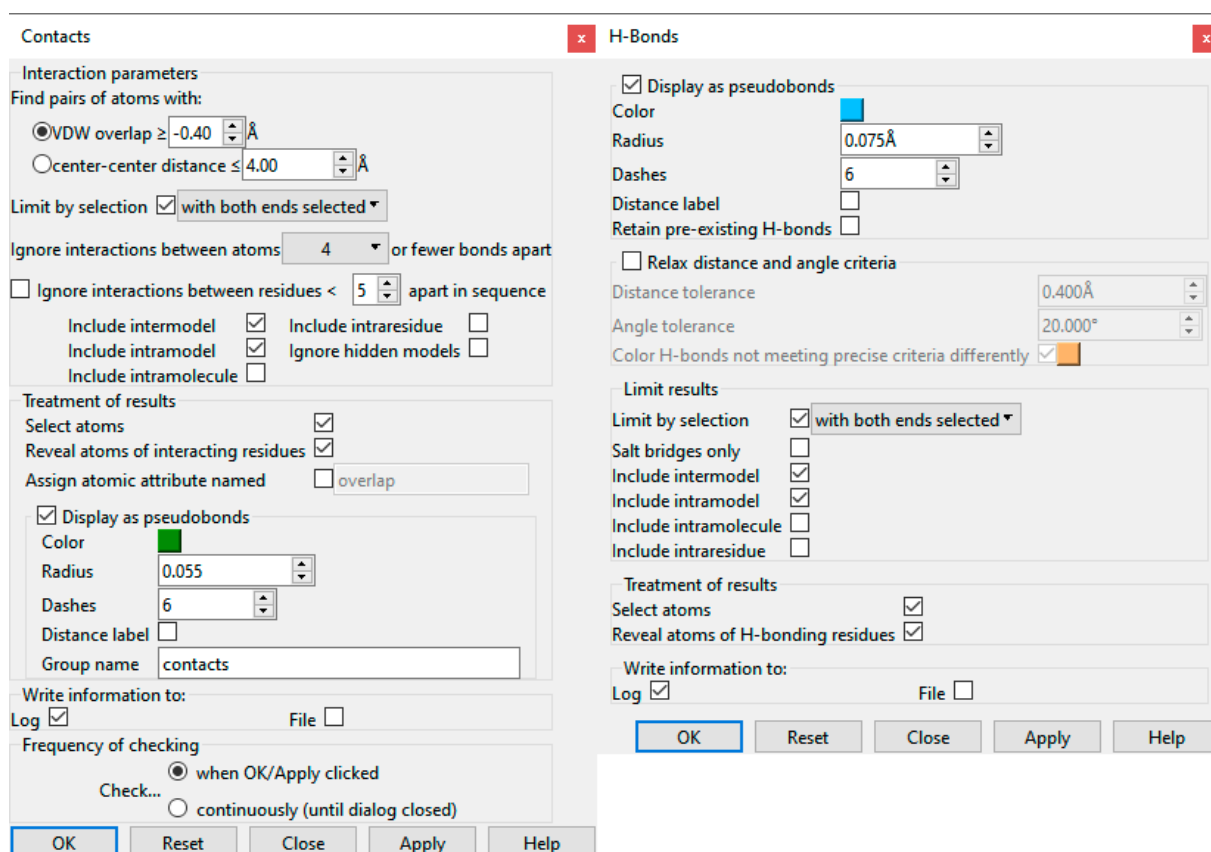


Figure S2. Settings for the Contacts and H-bonds tools in ChimeraX used for the detailed analysis of interactions between sense and antisense peptides. The Contacts tool (<https://www.cgl.ucsf.edu/chimerax/docs/user/commands/clashes.html>) parameters were configured to identify pairs of atoms with van der Waals (VDW) overlap ≥ -0.40 Å or center-center distances ≤ 4.00 Å, ignoring interactions between atoms separated by 4 or fewer bonds. The H-bonds tool (<https://www.cgl.ucsf.edu/chimerax/docs/user/tools/hbonds.html>) was configured to detect hydrogen bonds based on strict distance (≤ 0.400 Å) and angle ($\leq 20^\circ$) tolerances.

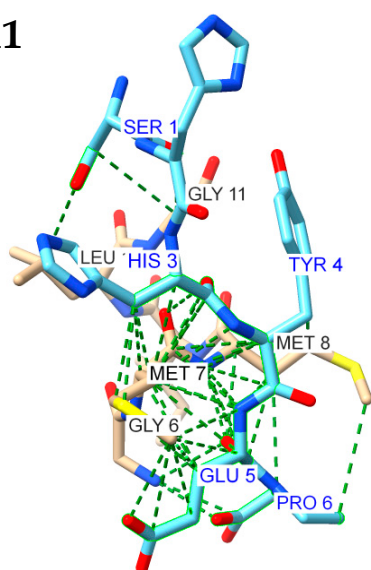
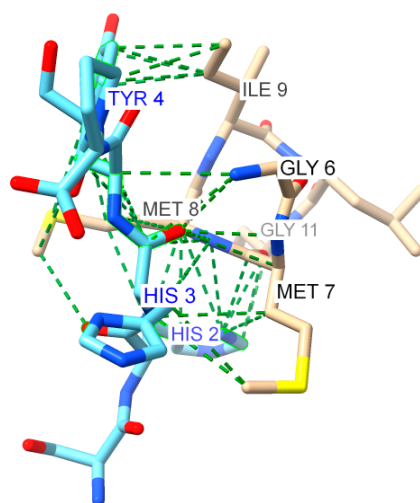
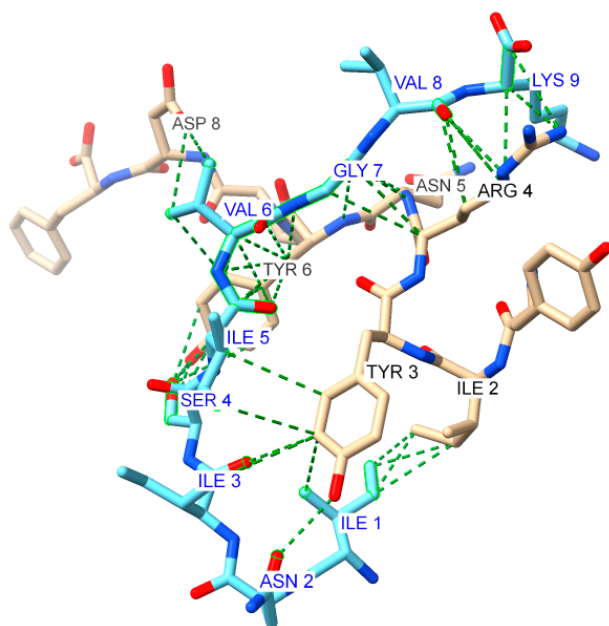
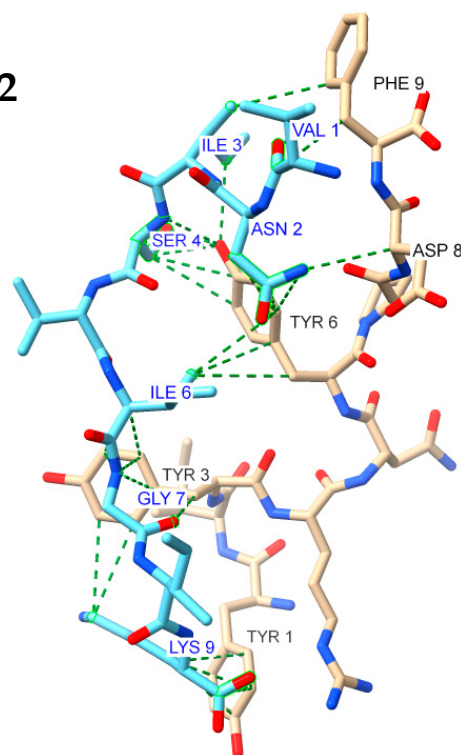
S1A1**S1A2****S2A1****S2A2**

Figure S3. Global docking simulations of the sense peptides S1 (GMMILG) and S2 (YIYRNYPDF) (tan models) with their respective antisense peptides (blue models). Docking simulations were performed using the HPEPDOCK web server, while visualization and contact analysis were carried out in UCSF ChimeraX (version 1.8). Interacting residues involved in the interface are labeled in black for the sense peptides and blue for the antisense peptides. Interactions are highlighted as green dashed pseudobonds, with atom colors distinguishing key elements: oxygen (red), nitrogen (blue), and sulfur (yellow).

Table S1. Summary of the major interactions for each antisense peptide with its sense counterpart, obtained using the Contacts and H-bonds tools in ChimeraX ([https://doi.org/ 10.1002/pro.3943](https://doi.org/10.1002/pro.3943)).

Peptide	Residue Pair	Interaction Type
S1A1	MET 2 - GLU 5	Hydrophobic interactions
	MET 2 - HYS 3	Hydrophobic interactions
	MET 3 - TYR 4	Potential sulfur- π interaction
	MET 3 - GLU 5	Potential H-bond*
S1A2	MET 2/3 - HIS 2/3	Hydrophobic interaction
	ILE 4 - TYR4	Hydrophobic interaction; potential π - π stacking**
	MET 3 - TYR 4	Potential sulfur- π interaction
	MET 3 - HIS 3	Potential H-bond*
S2A1	ILE 2 - ILE 1	Hydrophobic interaction
	TYR 6 - SER 4	Hydrophobic interaction
	TYR 6 - VAL 6	Hydrophobic interaction
	TYR 3 - ILE 3	Hydrophobic interaction; potential π - π stacking**
S2A2	TYR 6 - SER 4	Hydrophobic interaction
	LYS 9 - TYR 1	Hydrophobic interaction
	TYR 6 - ASN 2	Hydrophobic interaction; potential electrostatic interaction
	LYS 9 - TYR 1	Hydrophobic interaction

* Hydrogen bonds can be inferred based on geometric criteria involving only the donor and acceptor atoms. In such cases, potential hydrogen bonds can be inferred by evaluating distances and angles between these heavy atoms, even in the absence of explicit hydrogen atoms. This approach allows for the identification of probable hydrogen bonds, but they should be interpreted with caution, considering the limitations of not modeling hydrogen atoms explicitly.

** Recent research suggests that π - π interactions involving non-aromatic groups in protein structures may have been underappreciated in computational models. Unlike classical π - π stacking between two aromatic rings, these interactions involve planar sp^2 -hybridized orbital systems, including backbone amides and the side chains of residues such as Asn, Gln, Glu, and Asp (<https://doi.org/10.7554/eLife.31486>).

Table S2. Summary of the major interactions for each antisense peptide with AtxA, obtained using the Contacts and H-bonds tools in ChimeraX (<https://doi.org/10.1002/pro.3943>).

Peptide	Residue Pair	Interaction Type
S1A1	TYR 21 - HIS 3	π - π stacking, potential H-bond*
	VAL 30 - GLU 5	Hydrophobic, potential electrostatic
	ASP 48 - TYR 4	π - π stacking, potential H-bond
	CYS 44 - TYR 4	Potential sulfur- π ,** potential H-bond
S1A2	PHE 5 - TYR 4	π - π stacking
	ILE 9 - TYR 4	Hydrophobic
	GLY 6 - HIS 2	Potential H-bond
	PHE 96 - TYR 4	π - π stacking
	CYS 44 - TYR 4	Sulfur- π
S2A1	ARG 108 - ILE 1	Hydrogen bond
	LYS 37 - VAL 8	Hydrophobic
	TYR 110 - VAL 8	Hydrophobic
	ASN 109 - ASN 2	Potential H-bond
S2A2	LYS 9 - TYR 110	Hydrogen bond
	TYR 105 - VAL 1	Hydrophobic
	LEU 114 - ILE 8	Hydrophobic
	ARG 108 - ILE 3	Potential H-bond

* While traditionally hydrogen bonding is associated with electronegative atoms such as nitrogen, oxygen, and fluorine, studies have shown that carbon atoms can also engage in hydrogen bonding interactions. Under certain circumstances, methyl groups and other sp^3 hybridized carbons can form weak $CH\cdots O$ interactions and thus contribute to the stability and dynamics of protein structures (<https://doi.org/10.1002/prot.24724>; [https://doi.org/DOI: 10.1021/acs.jcim.2c00015](https://doi.org/DOI:10.1021/acs.jcim.2c00015)).

** Sulfur- π interactions are a type of non-covalent interaction that occurs between the sulfur atom of sulfur-containing amino acids (such as cysteine and methionine) and the aromatic rings of amino acids like tyrosine. Sulfur is one of the most polarizable atoms in proteins, which allows it to engage effectively with the electron cloud of the aromatic ring (<https://doi.org/10.1071/CH14598>).