In silico identification and chemical remodelling of tick protein epitopes for vaccine antigen development   https://doi.org/10.17952/37EPS.2024.P1119

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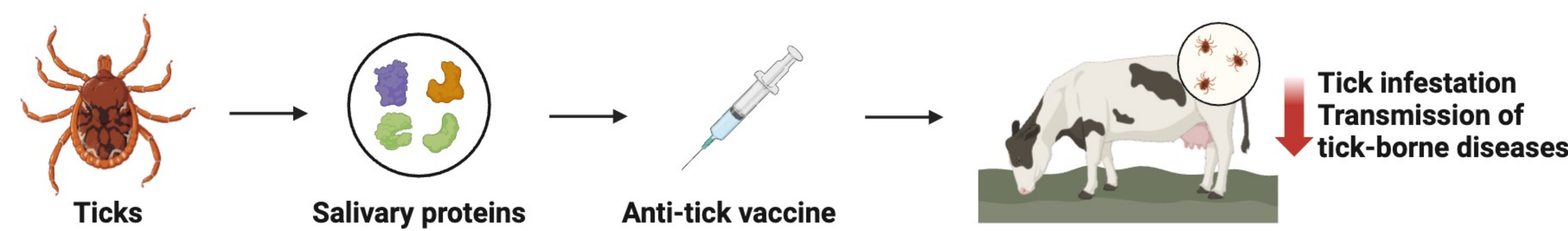
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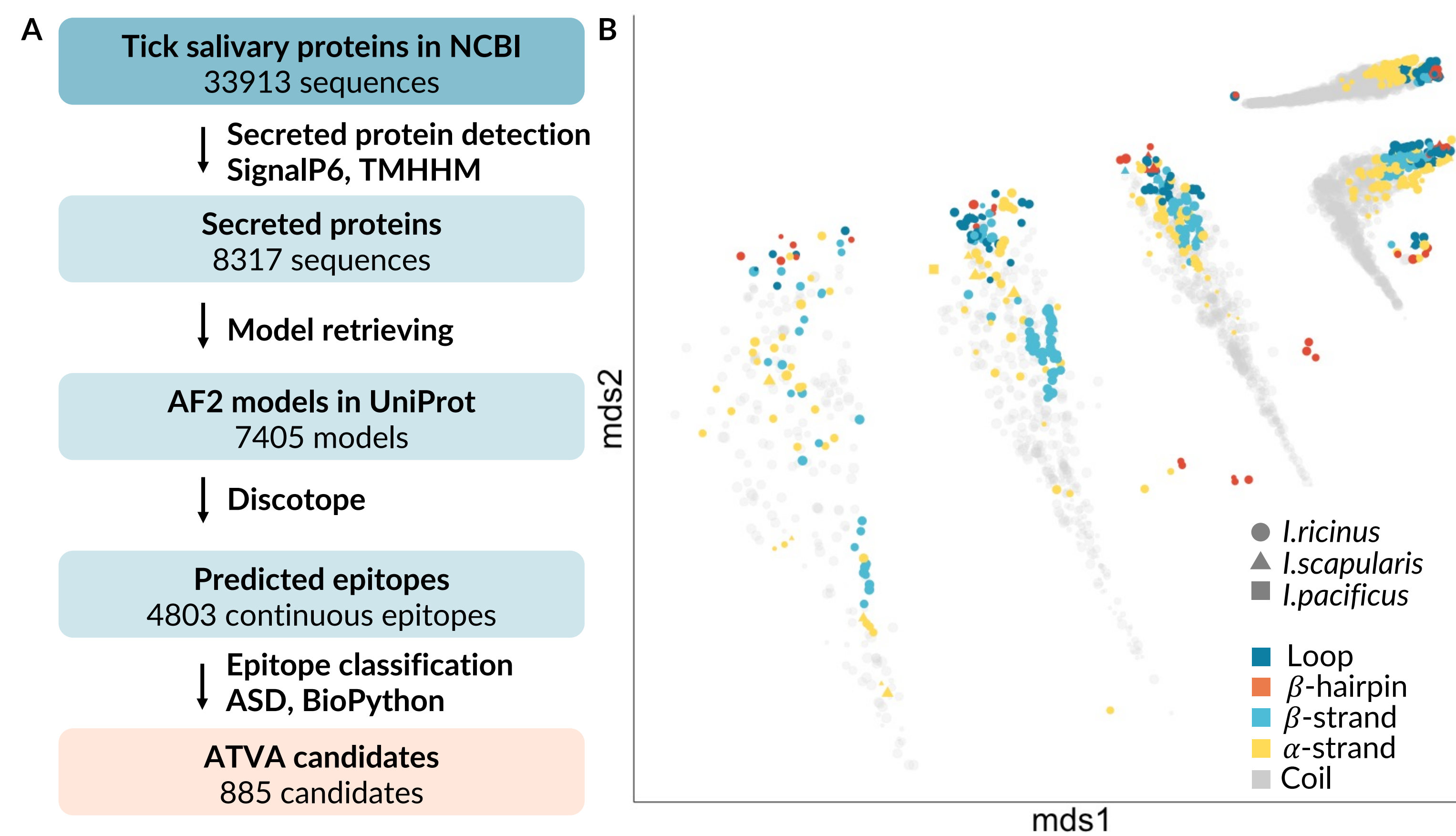[3]Institute of Parasitology, Biology Centre, Czech Academy of Sciences, České Budějovice, Czech Republic

Introduction

Ticks and tick-borne diseases are severe burdens for healthcare systems and for animal husbandry amounting to billions of dollars in economic losses worldwide. Due to climate change, ticks' habitats are expanding, which makes the need for novel ways of tick control as pressing as never before. One of the desirable strategies is the development of anti-tick vaccines to elicit acquired tick resistance[1]. The challenge in the development of such vaccines often lies in the inherently low immunogenicity and proteolytical stability of tick proteins, which they acquired through millions of years of evolution. Here we present a pipeline for the development of anti-tick vaccine antigens (ATVA) which utilizes AlphaFold2 structure modelling of tick proteins, in silico identification of antigenic epitopes by the protrusion-based algorithm[2], chemical remodelling and multimerization using the tick salivary lectin pathway inhibitor (TSLPI) as a model protein.
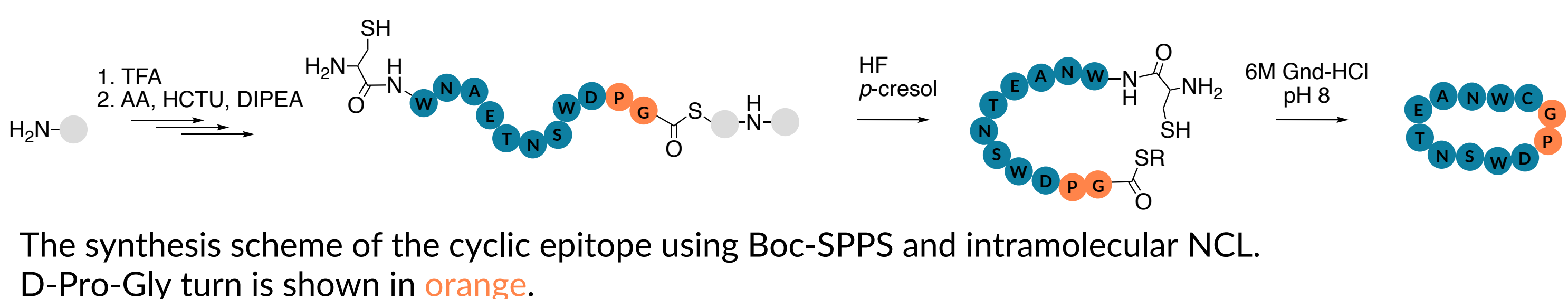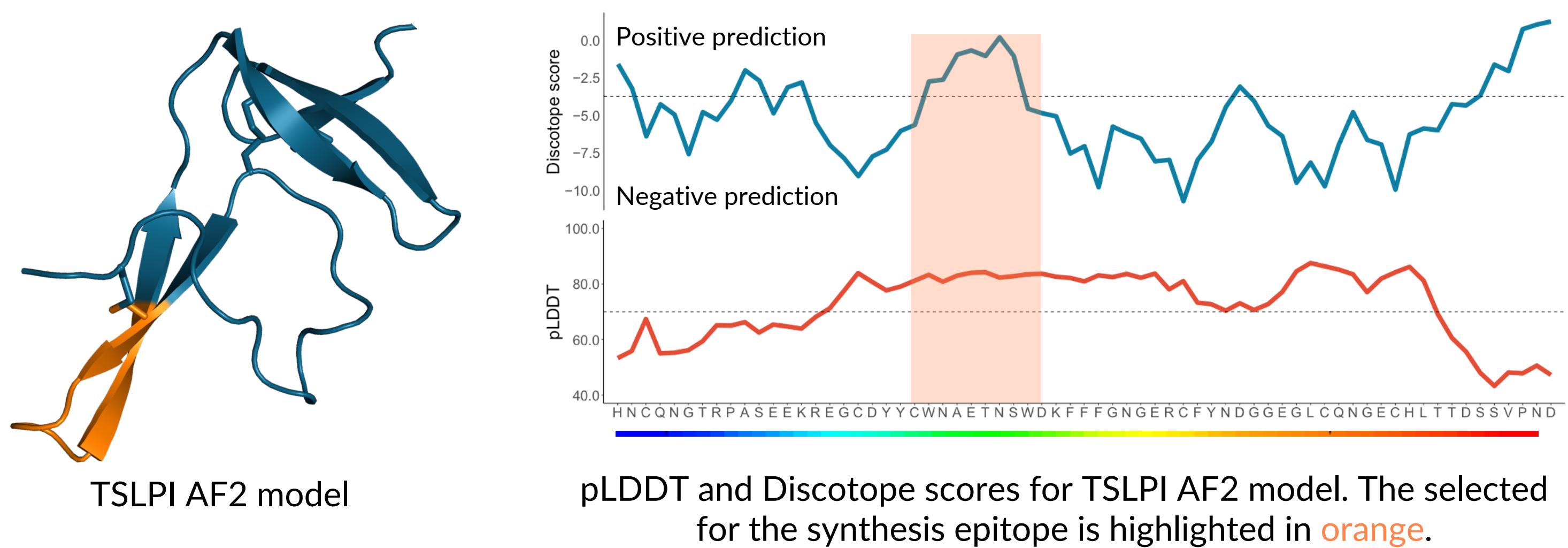

Tick infestation
Transmission of tick-borne diseases

Ticks → Salivary proteins → Anti-tick vaccine →

Bioinformatic pipeline allows mining of ATVA candidates

A
Tick salivary proteins in NCBI
33913 sequences
↓ Secreted protein detection SignalP6, TMHHM
Secreted proteins
8317 sequences
↓ Model retrieving
AF2 models in UniProt
7405 models
↓ Discotope
Predicted epitopes
4803 continuous epitopes
↓ Epitope classification ASD, BioPython
ATVA candidates
885 candidates

B
- I.ricinus
▲ I.scapularis
■ I.pacificus
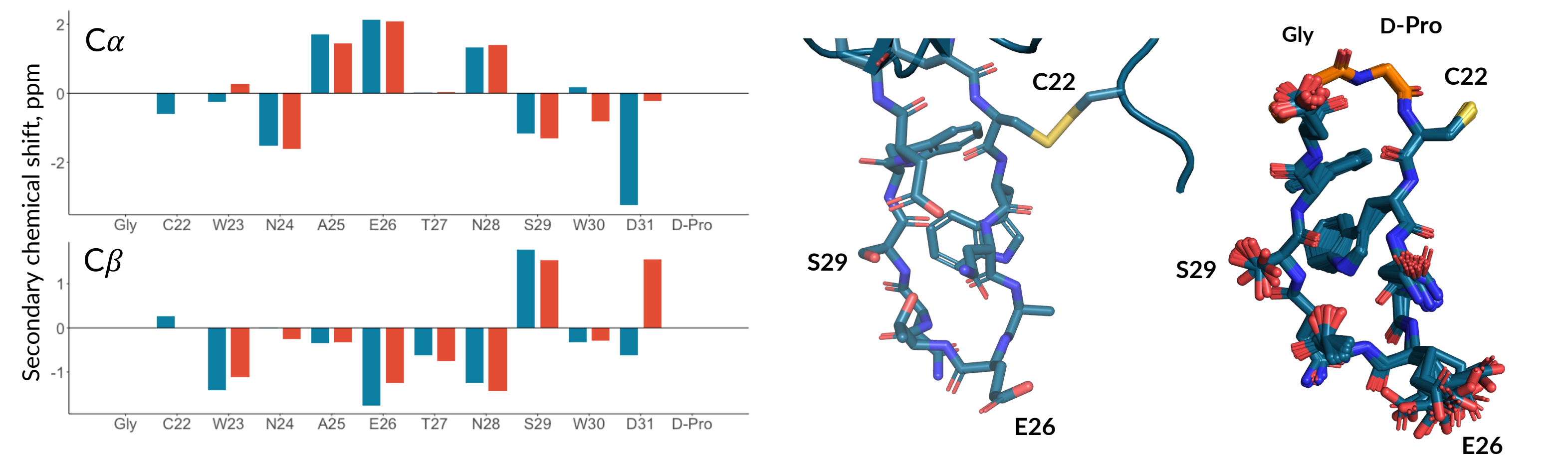
- Loop
- β-hairpin
- β-strand
- α-strand
- Coil

A. The pipeline for ATVA detection. B. The 2D representation of the epitope structural space based on amplitude spectrum distance (ASD)[3].

TSLPI contains a prospective epitope for ATVA

Tick salivary Lectin Pathway Inhibitor (TSLPI) from Ixodes Scapularis blocks the lectin pathway and facilitates transmission of Borrelia burgdorferi[4].

TSLPI AF2 model

pLDDT and Discotope scores for TSLPI AF2 model. The selected for the synthesis epitope is highlighted in orange.

The synthesis scheme of the cyclic epitope using Boc-SPPS and intramolecular NCL. D-Pro-Gly turn is shown in orange.

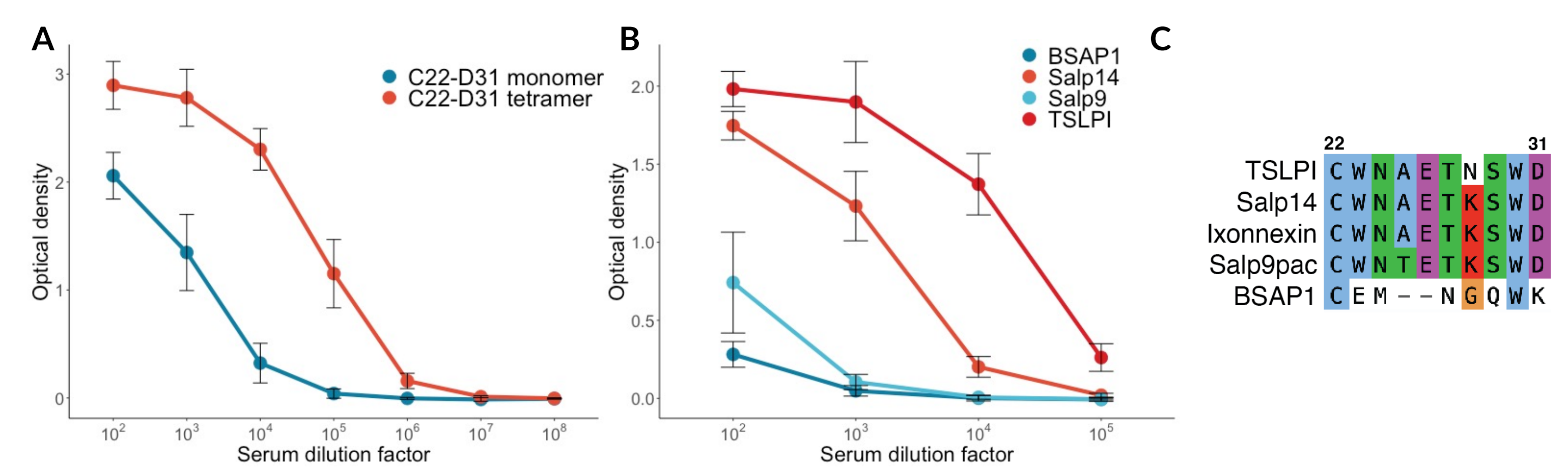## Synthetic epitope mimics the parental TSLPI

Comparison of secondary chemical shifts for full-length TSLPI and the cyclic epitope.

C22-D31 region in the TSLPI AF2 model (left) and overlay of 20 lowest-energy NMR structures of the cyclic epitope (right). D-Pro-Gly turn is shown in orange.

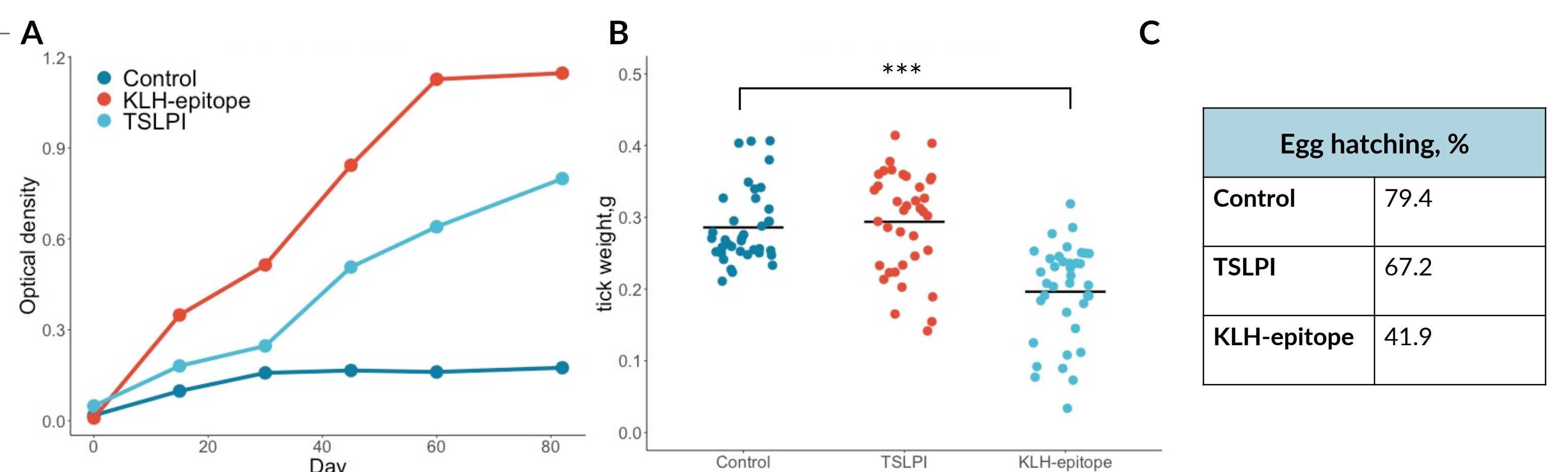## Epitope is multimerized for higher immunogenicity

Ser   Lys

R1:   R2:

23WNAETNSWD(D-P)GC22
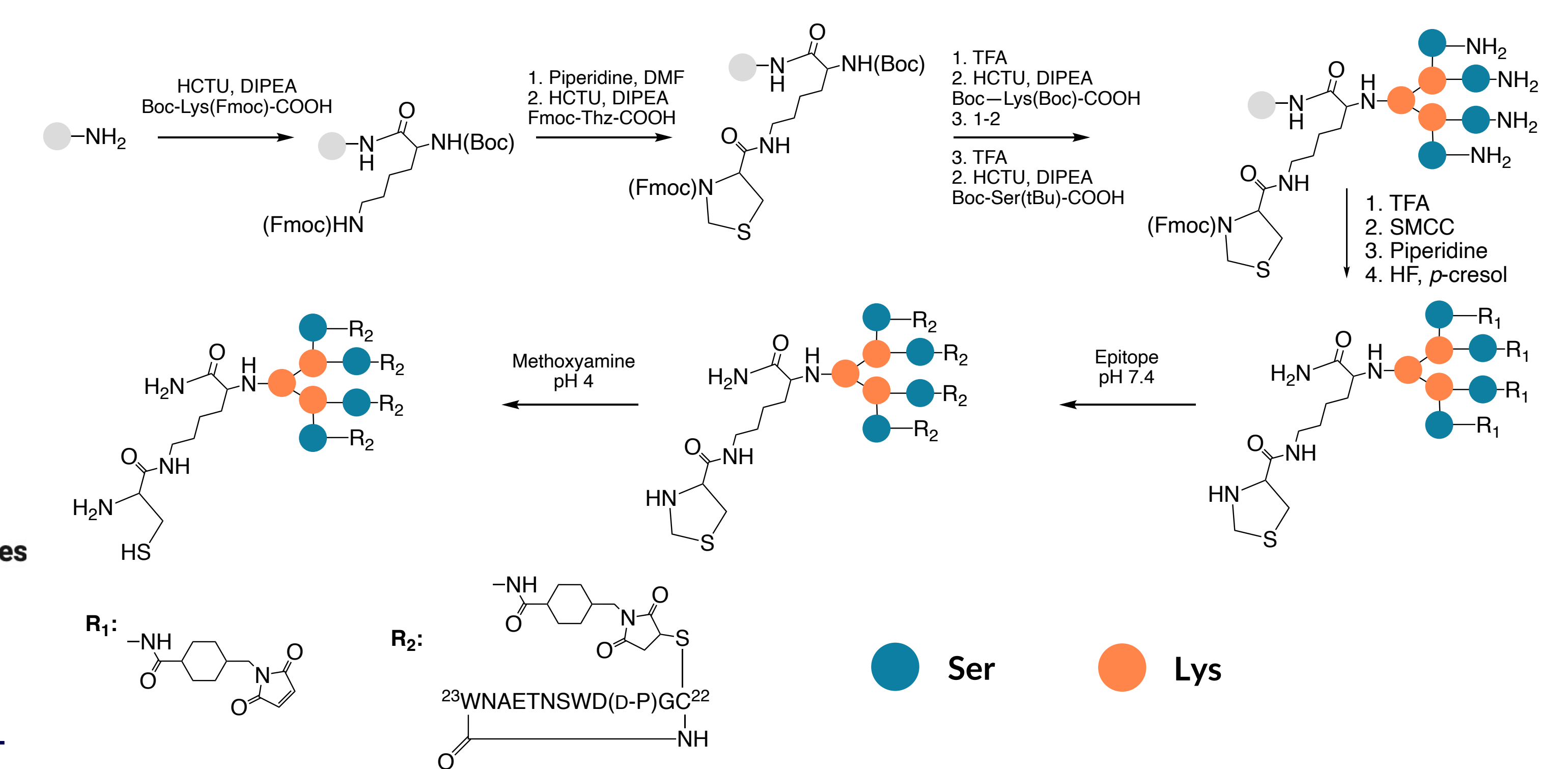
Synthesis of the tetrameric cyclic epitope coupled to a lysine wedge. SMCC, R1: succinimidyl-4-(N-maleimidomethyl)cyclohexane-1-carboxylate

Synthetic epitopes elicit specific immune response

A C22-D31 monomer, C22-D31 tetramer
B BSAP1, Salp14, Salp9, TSLPI
C

	22									31
TSLPI	C	W	N	A	E	T	N	S	W	D
Salp14	C	W	N	A	E	T	K	S	W	D
Ixonnexin	C	W	N	A	E	T	K	S	W	D
Salp9pac	C	W	N	T	E	T	K	S	W	D
BSAP1	C	E	M	–	–	N	G	Q	W	K

A. Level of TSLPI-specific antibodies in sera samples of mice vaccinated with KLH-peptide conjugates. **B.** Cross-reactivity of antibodies against tick proteins in sera samples of mice vaccinated with tetrameric cyclic epitope. **C.** Section of multiple sequence alignment of related to TSLPI proteins from I.scapularis.

A Control, KLH-epitope, TSLPI
B ***
C

	Egg hatching, %
Control	79.4
TSLPI	67.2
KLH-epitope	41.9

Results experiments with naïve rabbits (control), immunized with the parent TSLPI and tetrameric-cyclic C22-D31 TSLPI epitope (KLH-epitope) challenged with I.ricinus ticks. The level of antibodies in rabbit sera (A), tick weight (B) and egg hatching percentage (C).

Conclusions

- AlphaFold and structure-based epitope prediction algorithms allows fast identification of ATVB
- The predicted epitope from I.Scapularis was chemically synthesised and modified to structurally mimic the parental protein
- Tertramerized TSLPI epitope elicits higher TSLPI-specific immune response in mice compared to the monomeric epitope
- Immunisation of rabbits with the tetrameric epitope might affect tick feeding and reproduction

References
1. J. de la Fuente, et al., Vaccine 2017, 35, 5089–5094
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3. C. Galiez and F. Coste, BMC Bioinformatics 2015, 16,256
4. Schuijt et al., Cell Host & Microbe 2011, 10, 2, 136-146