Engineered synthetic virus-like particles and their use in antigen delivery

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Nanoparticle vaccine against *Streptococcus Pneumoniae*

Synthetic Virus-Like Particles (SVLPs) are a fully synthetic, modular nanoparticle platform that is highly immunogenic and capable of eliciting both antibody- and cell-mediated immune responses without the need for additional adjuvants.[1,2] Composed of self-assembling lipopeptide monomers (LPBs) that activate both innate and adaptive immunity,[1-3] SVLPs offer versatile applications, including vaccines for viral and bacterial infections, cancer immunotherapies, and allergy treatments.

Here, we introduce V-212, a nanoparticle vaccine targeting *Streptococcus pneumoniae* (*Spn*), a significant global health threat, particularly for children, the elderly, and immunocompromised individuals.[4] Current *Spn* vaccines are limited by their reliance on the bacterial capsular polysaccharide, which varies across more than 90 serotypes, reducing their overall efficacy.[5]

V-212 addresses these limitations by leveraging the SVLP platform. Through bioinformatics, screenings, and reverse engineering of convalescent patient sera, we identified three conserved epitopes (EP1, EP2, EP3) from *Spn* surface proteins. To enhance physicochemical properties and immunogenicity, two of these peptides were combined into a branched peptide and attached to one SVLP, while the third peptide was conjugated to a separate SVLP. The resulting V-212, a mixture of these two SVLPs, displays a homogenous particle distribution. In lethal sepsis models in mice, V-212 achieved 100% survival against *Spn* Serotype 3, with no detectable bacteria in blood on days 3 and 6. Additionally, vaccine-induced antibodies demonstrated enhanced bacterial clearance by human immune cells in vitro. V-212 is currently under preparations for a Phase 1 clinical trial.

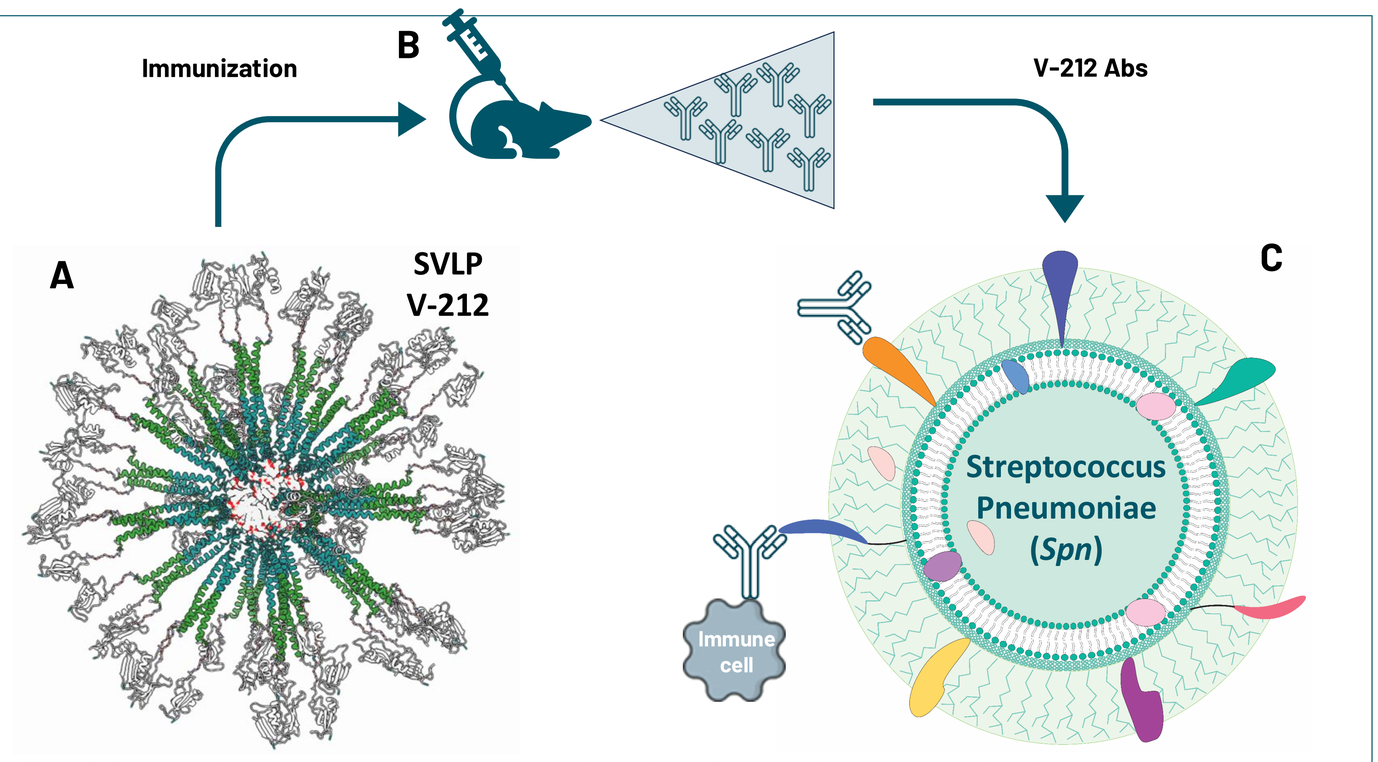


Figure 1: A Computational model of a traditional SVLP presenting antigens. **B** Immunization of animal species with SVLPs showing epitope specific antibodies. **C** Schematic representation of *Spn* showing the capsule and surface proteins. **C** V-212 Abs are binding to *Spn* surface proteins promoting bacterial clearance.

Virometix technology: Lipopeptide block (LPB)

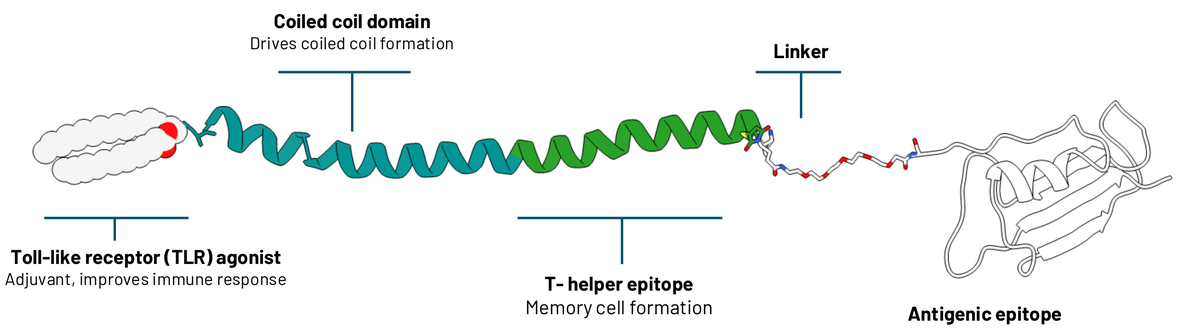


Figure 2: Schematic representation of the lipopeptide block showcasing the different elements

LPB Synthesis – Pseudoprolines as key elements in synthesis

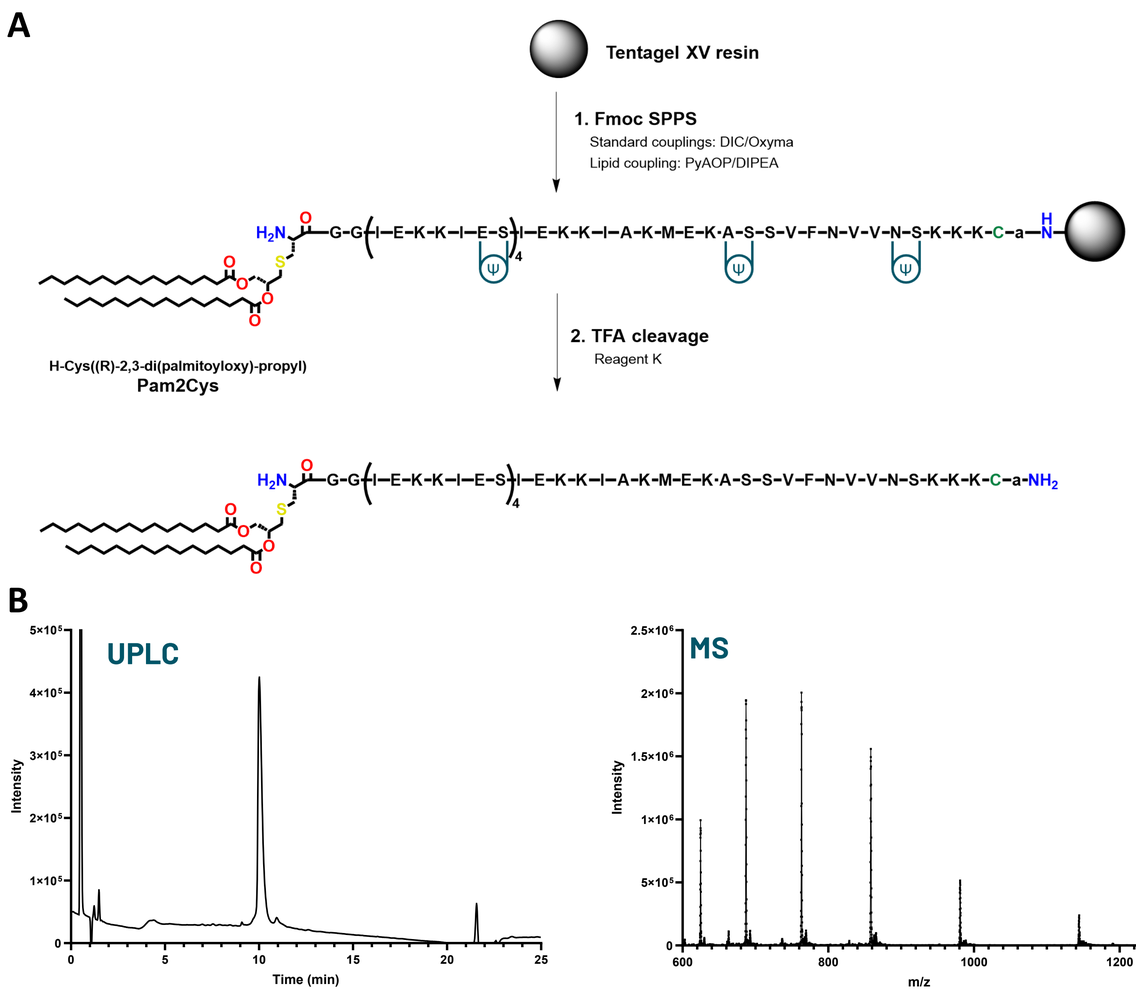
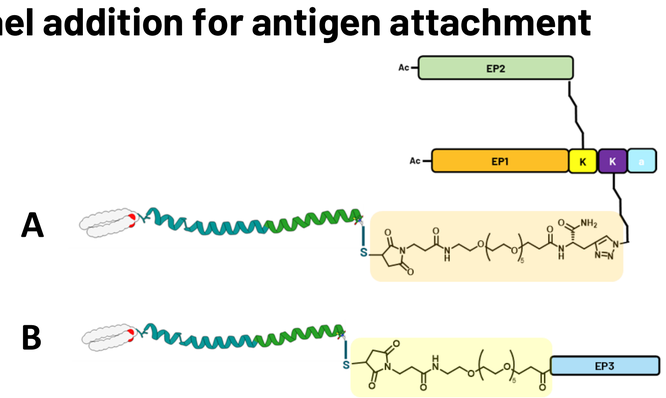


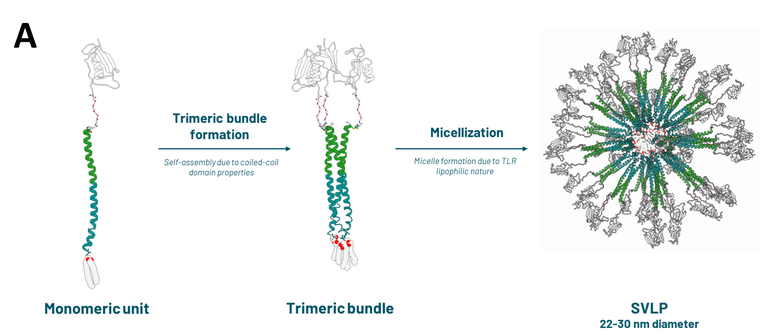
Figure 3: A Schematic representation of the LPB block. The synthesis was performed in Tentagel XV resin using pseudoproline building blocks spaced approximately every 5 AA. The TLR agonist Pam2Cys was added with PyAOP. The peptide was finally cleaved from the resin using Reagent K and purified. **B** LPB analysis showing the UV trace on a UPLC and the MS pattern of the LPB.

V-212 CuAAC and Thiol-Michael addition for antigen attachment

Figure 4: Antigens and linkers used in the construction of V-212. **A** Antigen conjugation of a branch epitope to the LPB that contains a linker with an alkyne moiety, the conjugation was done using copper catalysed click chemistry (CuAAC). **B** Antigen conjugation through Michael-thiol addition



V-212 forms sharp monodisperse particles



Z-Average: 28.11 ± 0.01 nm
Polydispersity Index: 0.04 ± 0.01

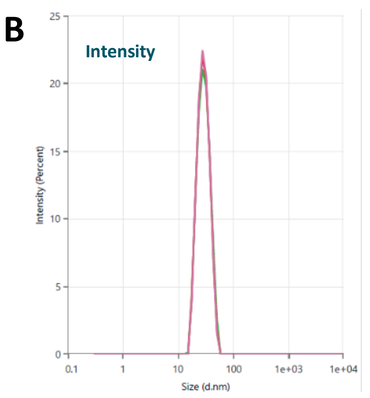


Figure 5: The LPB forms SVLPs through coiled coil assembly and micellization**. A** Schematic representation of SVLP formation showing the trimeric coiled coil formation and the micellization. **B** Particle distribution measured for V-212 with dynamic light scattering (DLS) and its values.

V-212 provides protection in Lethal Sepsis models

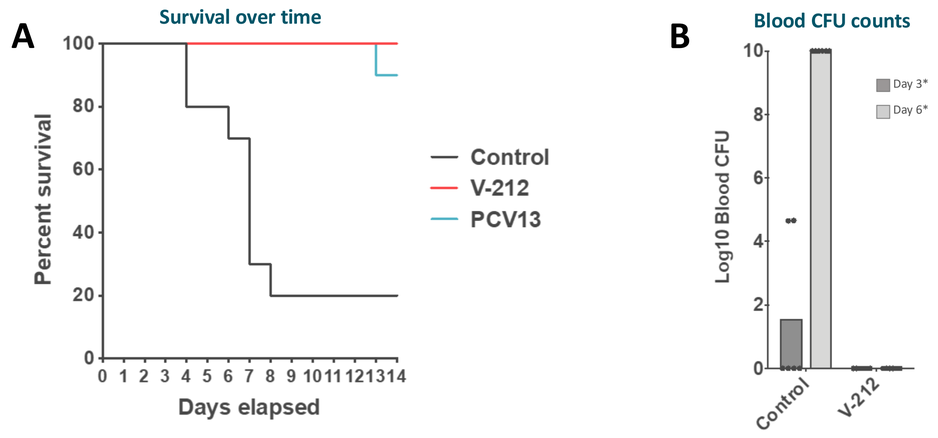


Figure 6: Results from a lethal sepsis model comparing PCV13 and V-212 against Spn Ser 3. **A** Survival of the groups overtime after being challenged on day 42 with $5x10^5$ CFU of Ser3. **B** Difference of colony forming units (CFU) in the control *vs* V-212 group

V-212 Abs increase phagocytic uptake

Figure 7: Measurements of phagocytic uptake using FACS. **A** schematic representation of the phagocytic uptake promote by V-212 Abs. **B** Example of a FACS analysis showing differences in fluorescence with V-212 group. **C** Difference in Uptake % for *Spn* Ser 4 and 19F.

V-212 Status and next steps

Tox studies
V-212 safe profile
Completed

Vaccine **mechanism of action**
Undergoing*

GMP manufacturing
50g scale
Completed

Clinical trial Phase 1
in preparation

References

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[5] Lagousi, T., Basdeki, P., Routsias, J., & Spoulou, V. (2019). Novel protein-based pneumococcal vaccines: assessing the use of distinct protein fragments instead of full-length proteins as vaccine antigens. Vaccines, 7(1), 9.