Deimmunization of peptidoglycan hydrolases for therapeutic treatment of systemic *S. aureus* infections

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Aim: The goal of this project is to deimmunize highly active phage-derived peptidoglycan hydrolases (PGHs) for future application in humans with systemic *S. aureus* infections.

Staphylococcus aureus

- Gram-positive
- Opportunistic pathogen
  - Food poisoning
  - Life-threatening infections leading to bacteremia
  - Main cause of hospital acquired diseases
- Antibiotic resistant strains
  - Methicillin-resistant *S. aureus* (MRSA)
  - Vancomycin-resistant *S. aureus* (VRSA)

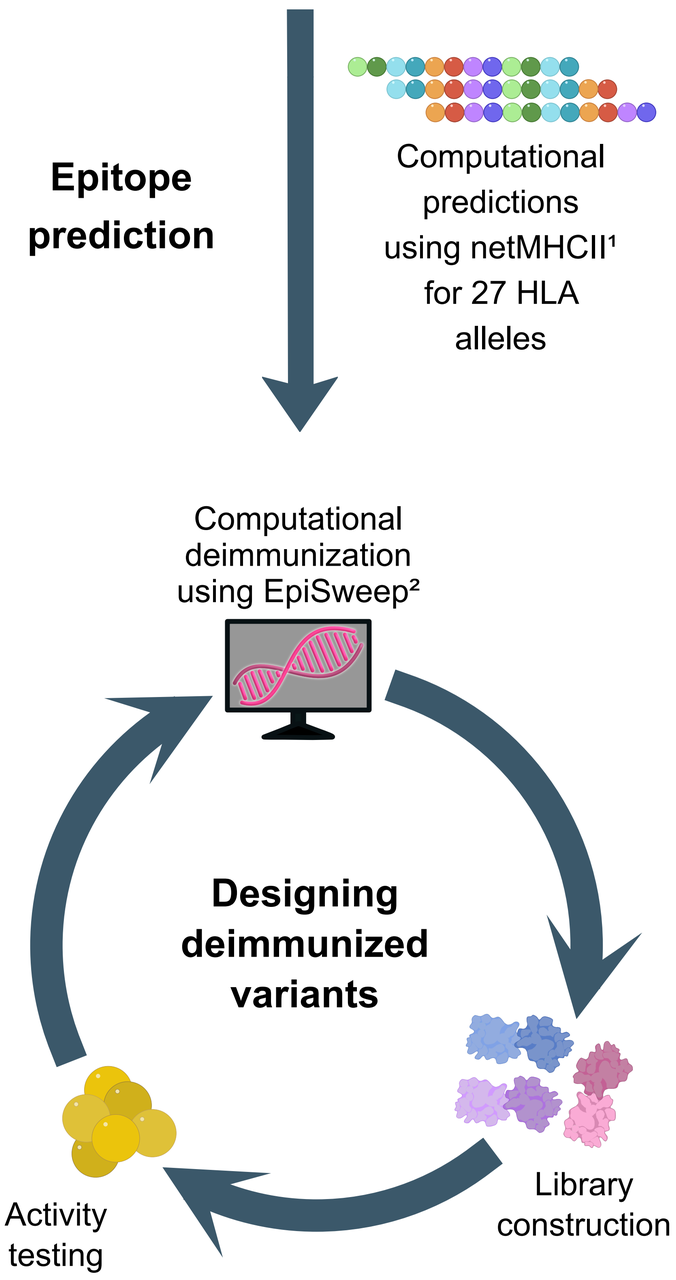
Endolysins

- Phage-derived PGHs
- Modular architecture → easy to engineer
  - Cell wall binding domain (CBD)
  - Enzymatically active domain (EAD)
- Active against antibiotic resistant strains
- Highly specific for target organism
- Rapid mode of action
- Independent of bacterial metabolism

Immune system - T cell epitopes

- Short peptide fragments (10-28aa)
- Linear epitopes
- Key upstream role in antidrug antibody response
- Established deimmunization methods[1]
- MHC highly polymorphic
  - HLA class I: > 25'000 alleles
  - HLA class II: > 10'000 alleles

Workflow

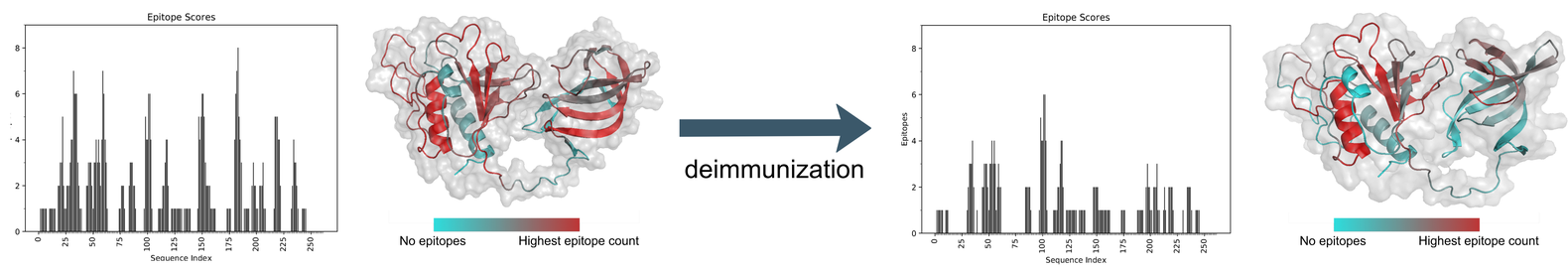


***Ex vivo* immunogenicity testing**

Cultured ELISpot assays with PBMCs from healthy human donors

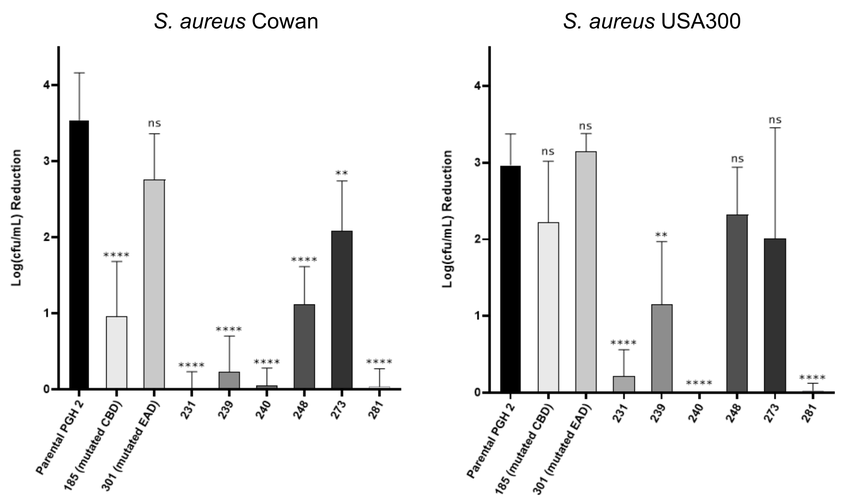
***In vivo* immunogenicity & efficacy testing**

Epitope prediction



Predicted eitopes for the parental protein and computationally deimmunized variant. Epitopes were predicted with netMHCII¹ and deimmunization was performed using EpiSweep². Epitopes were predicted for 27 MHCII alleles in order to cover the broad human population by including alleles dominant for all ethnicities.

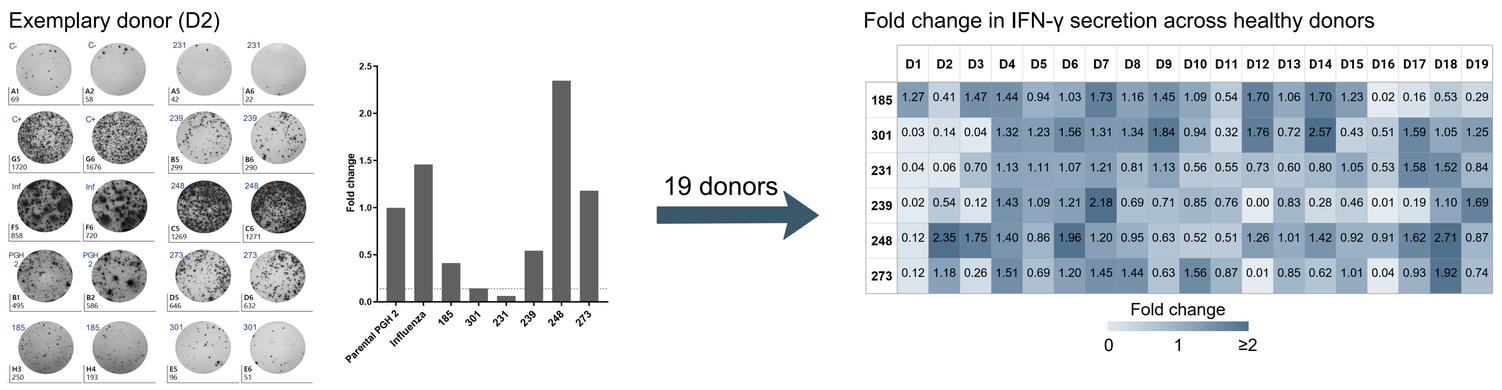
Activity testing



ns, $p > 0.05$; *, $p \leq 0.05$; **, $p \leq 0.01$; ***, $p \leq 0.001$

High throughput activity screenings were preformed to identify staphylolytic variants in the library of deimmunized PGHs in human serum. Promising canditates were assessed against *S. aureus* Cowan (MSSA) and *S. aureus* USA300 (MRSA) in time kill assays in human serum for one hour. The variants were compared to the unmutated parental protein. A non significant difference in log(CFU/mL) reduction compared to the parental protein indicates retained enzymatic activity despite the introduction of deimmunizing mutations.

Immunogenicity testing



	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12	D13	D14	D15	D16	D17	D18	D19
185	1.27	0.41	1.47	1.44	0.94	1.03	1.73	1.16	1.45	1.09	0.54	1.70	1.06	1.70	1.23	0.02	0.16	0.53	0.29
301	0.03	0.14	0.04	1.32	1.23	1.56	1.31	1.34	1.84	0.94	0.32	1.76	0.72	2.57	0.43	0.51	1.59	1.05	1.25
231	0.04	0.06	0.70	1.13	1.11	1.07	1.21	0.81	1.13	0.56	0.55	0.73	0.60	0.80	1.05	0.53	1.58	1.52	0.84
239	0.02	0.54	0.12	1.43	1.09	1.21	2.18	0.69	0.71	0.85	0.76	0.00	0.83	0.28	0.46	0.01	0.19	1.10	1.69
248	0.12	2.35	1.75	1.40	0.86	1.96	1.20	0.95	0.63	0.52	0.51	1.26	1.01	1.42	0.92	0.91	1.62	2.71	0.87
273	0.12	1.18	0.26	1.51	0.69	1.20	1.45	1.44	0.63	1.56	0.87	0.01	0.85	0.62	1.01	0.04	0.93	1.92	0.74

Ex vivo immunogenicity assay with peripheral blood mononuclear cells (PBMCs) of healthy donors exposed to unmutated and deimmunized protein variants for 7 days. IFN-γ secretion is measured for 24h after the co-culturing of one week using anti-IFN-γ coated enzyme-linked immunospot (ELISpot) plates. The substrate is unsolubale and therefore leads to visible spots on the surface of the well. Each spot corresponds to an individual cytokine-secreting cell. Immuneresponse is highly donor-dependent and data from 19 donors is combined into a heatmap showing the fold change in IFN-γ secretion of the deimmunized variants compared to the parental PGH.

Outlook - *in vivo* studies

Most promising cadidates will be assessed for immunogenicity and efficacy in an HLA-DR*0401 transgenic mouse model

- **Immunogenicity study**: repeated protein injection followed by antibody monitoring
- **Efficacy study**: repeated weekly infection and treatment to assess advantage of antibody-evading deimmunized variants

Conclusion:

- PGHs are highly promising antimicrobials for therapeutic application
- Immunogenicity is a major problem associated with therapeutic proteins
- Combination of computational and experimental tools are most efficient for protein deimmunization

References:

[1] Kamilla Kjaergaard Jensen, et al. "Improved methods for predicting peptide binding affinity to MHC class II molecules." Immunology. 2018.

[2] Choi Yoonjoo, et al. "EpiSweep:Computationally driven reengineering of therapeutic proteins to reduce immunogenicity while maintaining function." Computational Protein Design. 2017.

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