A self-assembling and self-adjuvanting multiepitope peptide nanoparticle vaccine improves the efficacy and immunogenicity of Bacille Calmette-Guérin

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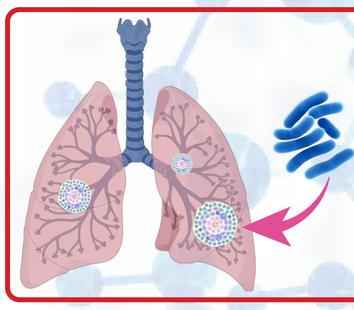
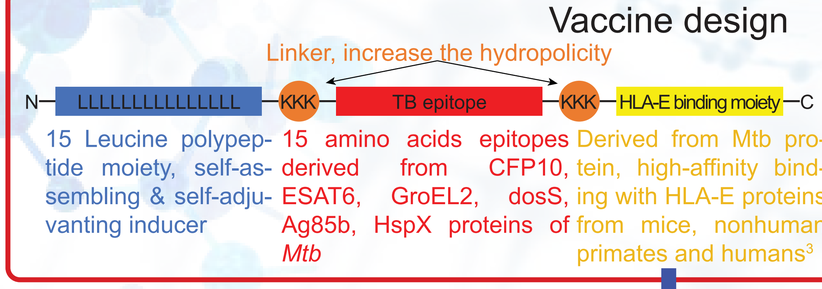
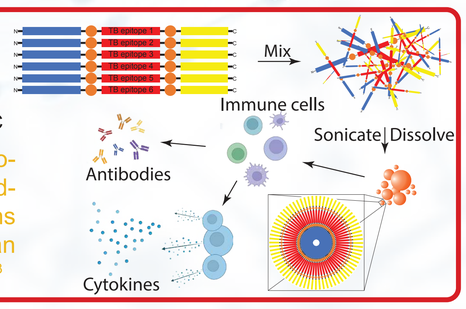
Background



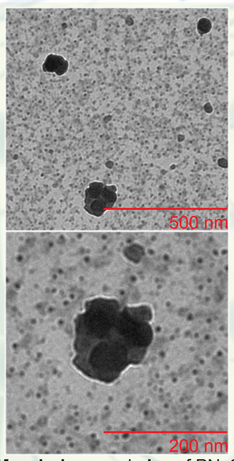
- TB is the 13th leading cause of death and the second leading infectious killer after COVID-19 (above HIV/AIDS)[1]
- The global burden of LTBI was 23.0%
- Multidrug-resistant TB (MDR-TB) remains a public health crisis and a health security threat.
- After one century, BCG still remains the only licenced TB vaccine
- Previously we discovered that a 15 leucine polypeptide moiety as a self-assembling and self-adjuvanting inducer of peptide-based vaccine[2]

Vaccine design

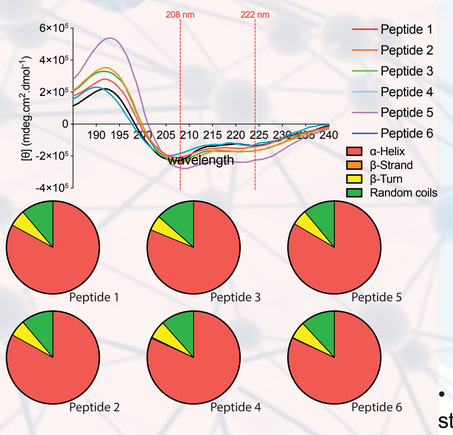




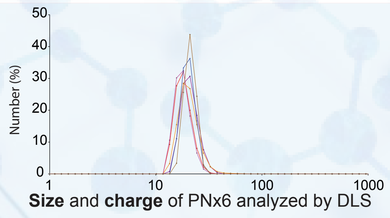
PNx6 physicochemical property characterization



Morphology and **size** of PNx6 analyzed by TEM



Secondary structure of PNx6 analyzed by CD

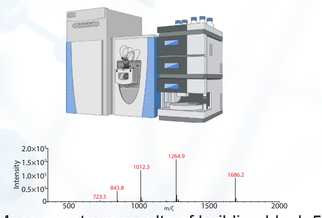


Size and **charge** of PNx6 analyzed by DLS

	Size (nm)	Zeta potential (mV)	α-helix %
Peptide 1	22 ± 4; 134 ± 70	29 ± 10	83.00
Peptide 2	34 ± 7; 162 ± 34	37 ± 6	83.10
Peptide 3	20 ± 7; 201 ± 46	14 ± 5	81.20
Peptide 4	22 ± 2; 141 ± 13	34 ± 8	81.80
Peptide 5	98 ± 37; 418 ± 192	46 ± 11	83.50
Peptide 6	10 ± 2; 61 ± 25	19 ± 8	81.90
PNx6	27 ± 6; 256 ± 36	48 ± 6	

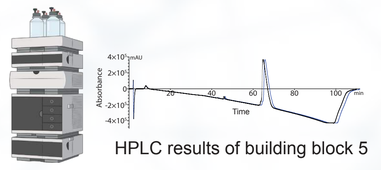
- All peptide building blocks for PNx6 have **α-helix** structure
- PNx6 are able to self-assemble into nanoparticles
- PNx6 nanoparticles are positively charged

Vaccine synthesis



Mass spectrum results of building block 5

- Fmoc solid-phase peptide synthesis
- 2-Chlorotrityl chloride resin
- All peptide building blocks were successfully synthesized
- Purity ≥ 95%



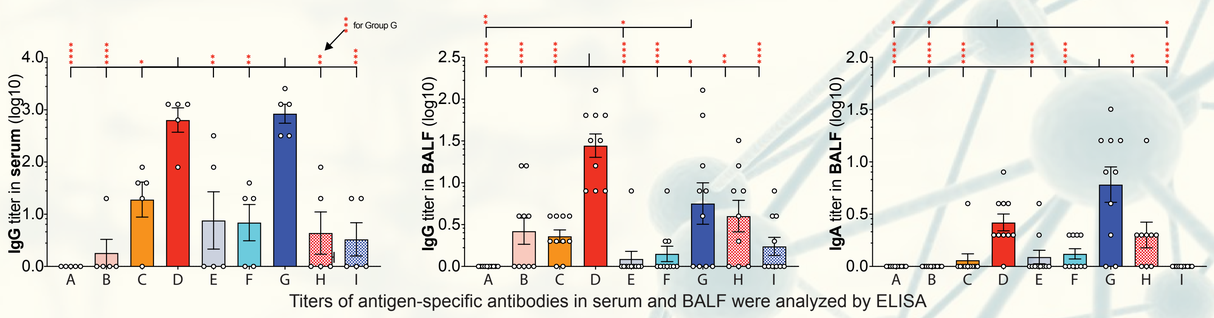
HPLC results of building block 5

Immunology evaluation of PNx6

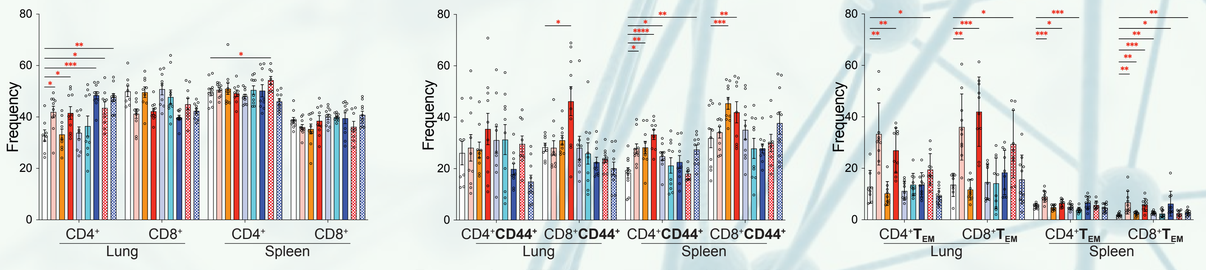
Group	Treatment	Mice No.	Dose (per mouse, per administration)	Legend
A	unvaccinated	n=10		
B	s.c. BCG	n=10	s.c. 1×10⁶ BCG (in 200 μL PBS) on Day 0	
C	s.c. PNx6	n=10	s.c. 50 μL (3mg/mL) PNx6 on Day 0, 14, 28 and 42	
D	s.c. BCG + s.c. PNx6	n=10	s.c. 200 μL BCG on Day 0 + s.c. 50 μL (3mg/mL) PNx6 on Day 14, 28 and 42	
E	i.n. BCG	n=10	i.n. 1×10⁶ BCG (in 40 μL PBS) on Day 0	
F	i.n. PNx6	n=10	i.n. 50 μL (3mg/mL) PNx6 on Day 0, 14, 28 and 42	
G	i.n. BCG + i.n. PNx6	n=10	i.n. 40 μL BCG on Day 0 + i.n. 50 μL (3mg/mL) PNx6 on Day 14, 28 and 42	
H	s.c. BCG + i.n. PNx6	n=10	s.c. 200 μL BCG on Day 0 + i.n. 50 μL (3mg/mL) PNx6 on Day 14, 28 and 42	
I	i.n. BCG + s.c. PNx6	n=10	s.c. 200 μL BCG on Day 0 + i.n. 50 μL (3mg/mL) PNx6 on Day 14, 28 and 42	

- s.c. BCG + s.c. PNx6 induced highest amount of IFNγ-secreting cells
- s.c. BCG + s.c. PNx6 induced significantly higher titer of antigen-specific antibodies than s.c. BCG
- s.c. BCG + s.c. PNx6 induced higher frequency of immune cells
- s.c. BCG + s.c. PNx6 induced significantly higher frequency of polyfunctional cytokine secreting T cells

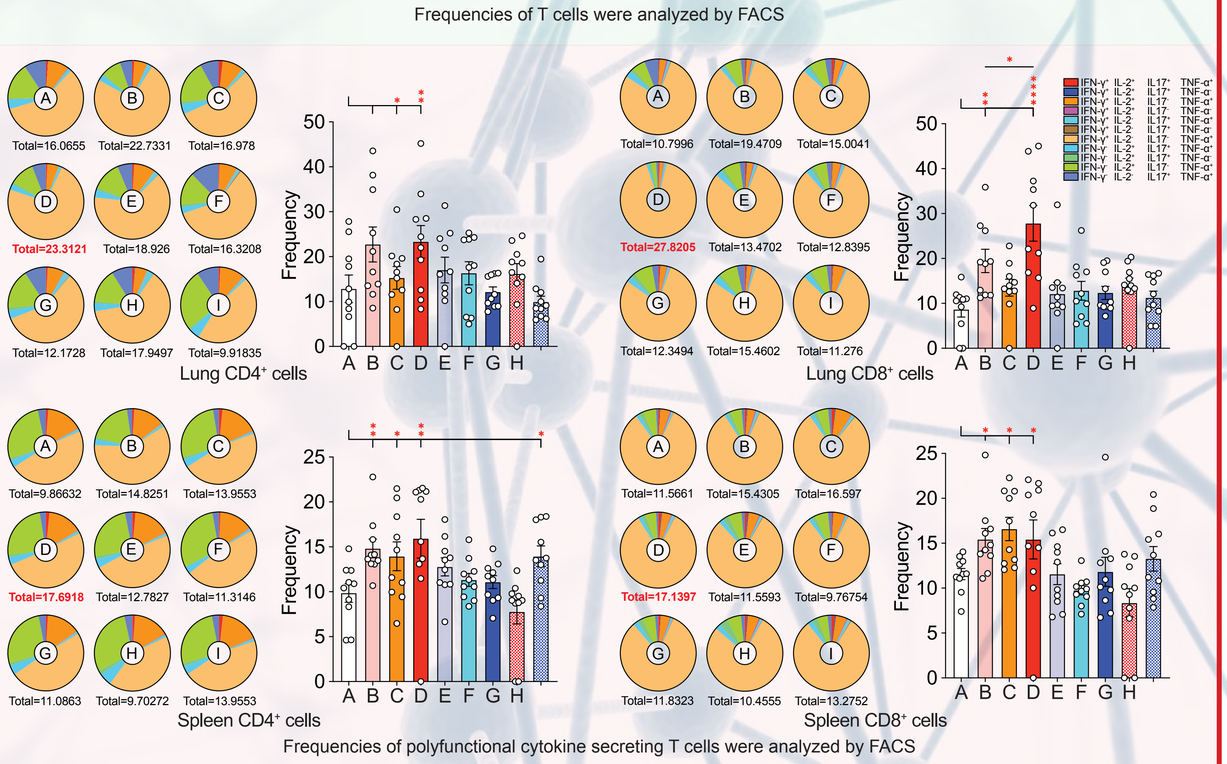
Effect of antigen stimulation on IFNγ secretion of PNx6 was analyzed by ELISpot



Titers of antigen-specific antibodies in serum and BALF were analyzed by ELISA

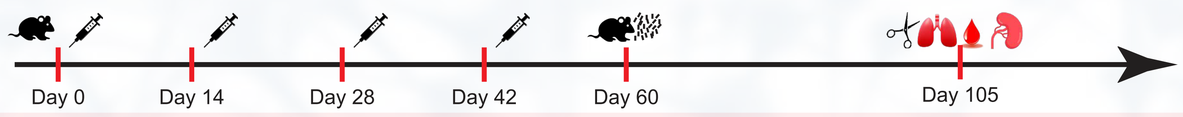


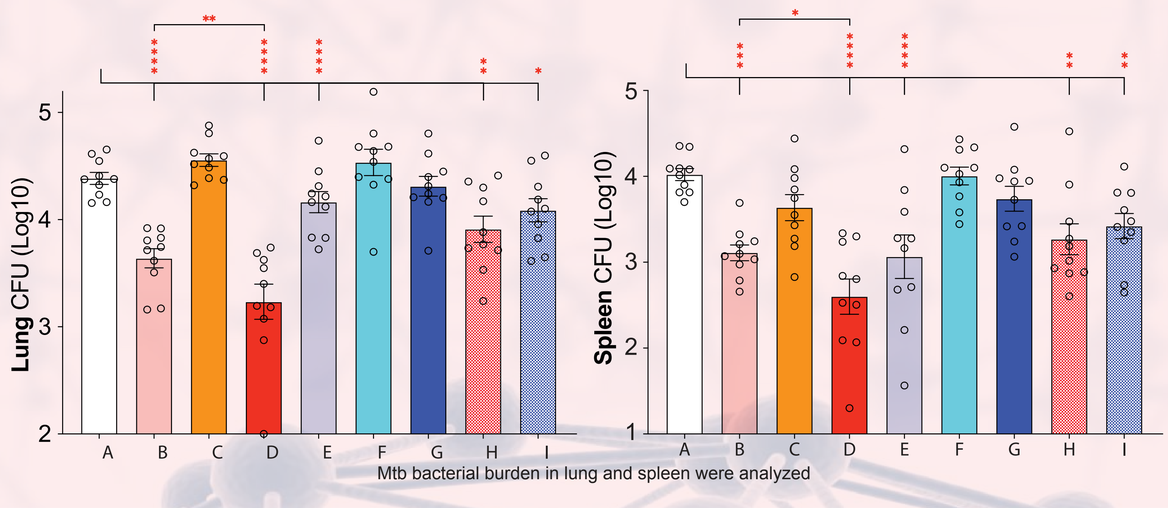
Frequencies of T cells were analyzed by FACS



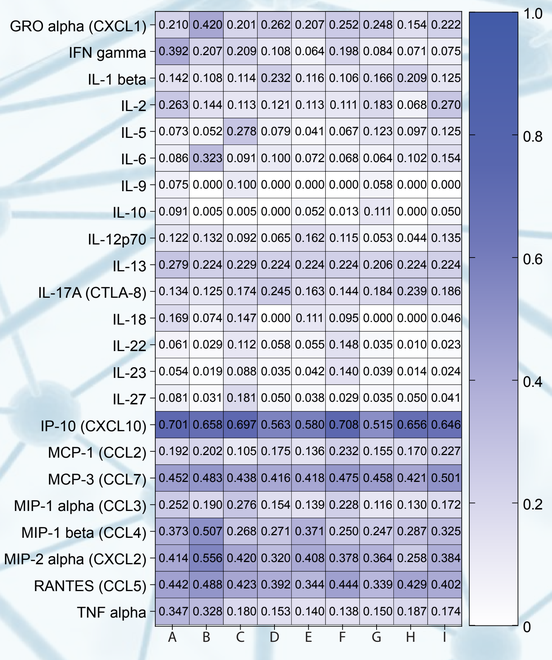
Frequencies of polyfunctional cytokine secreting T cells were analyzed by FACS

Protection efficacy evaluation of PNx6

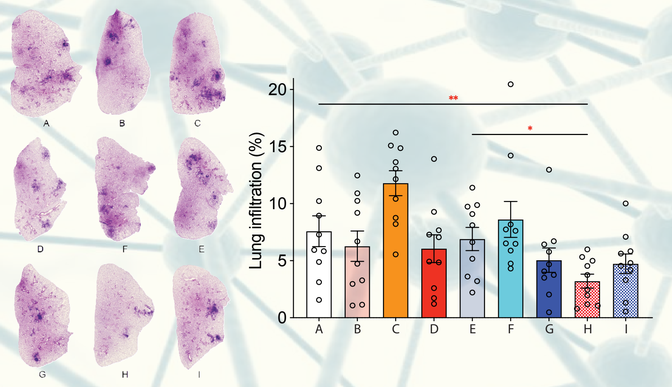




Mtb bacterial burden in lung and spleen were analyzed



Profile of cytokines and chemokines in serum



Lung damage was analyzed by the percentage of infiltration area

- s.c. BCG + s.c. PNx6 vaccinated mice have significantly lower bacterial burden in both lung and spleen than s.c. BCG only
- s.c. BCG + s.c. PNx6 vaccinated mice have less lung damage
- s.c. BCG + s.c. PNx6 vaccinated mice have lower titer of cytokines and chemokines in serum

Summary

- s.c. BCG + s.c. PNx6 vaccination induced much stronger immune response than s.c. BCG only
- s.c. BCG + s.c. PNx6 immunity demonstrated better protection efficacy than s.c. BCG vaccination only
- As previous study proved that the 15 leucine polypeptide moiety is non-cytotoxic, the safety of s.c. BCG + s.c. PNx6 vaccination should be similar to s.c. BCG
- More *Mtb* peptide epitopes and binding peptides could be incorporated into the PNx6 platform to produce a stronger booster vaccine for BCG

Advantages of PNx6

- Cold-chain and adjuvant independent
- Use of cross-species validated epitopes and binding peptides could facilitate potential clinical translation
- The modular building block approach allows for rapid, economical, and customized vaccine production
- The peptides can be stored and transported as freeze-dried solids at room temperature
- PNx6 can be prepared on-site by convenient two steps (dissolve, sonicate) within 2 minutes
- Infinite potential to optimize by including more peptide epitopes
- Produce customizable vaccine by incorporating specific peptide epitopes

Reference

1. Bagcchi, S. (2023). WHO's global tuberculosis report 2022. The Lancet Microbe 4, e20.
2. Skwarczynski, M., **Zhao, G.**, Boer, J.C., Ozberk, V., Azuar, A., Cruz, J.G., Giddam, A.K., Khalil, Z.G., Pandey, M., Shibu, M.A., et al. (2020). Poly(amino acids) as a potent self-adjuvanting delivery system for peptide-based nanovaccines. Science Advances 6, eaax2285. doi:10.1126/sciadv.aax2285.
3. Ruibal, P., Franken, K.L.M.C., van Meijgaarden, K.E., van Loon, J.J.F., van der Steen, D., Heemskerk, M.H.M., Ottenhoff, T.H.M., and Joosten, S.A. (2020). Peptide Binding to HLA-E Molecules in Humans, Nonhuman Primates, and Mice Reveals Unique Binding Peptides but Remarkably Conserved Anchor Residues. The Journal of Immunology, ji2000810. 10.4049/jimmunol.2000810.

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