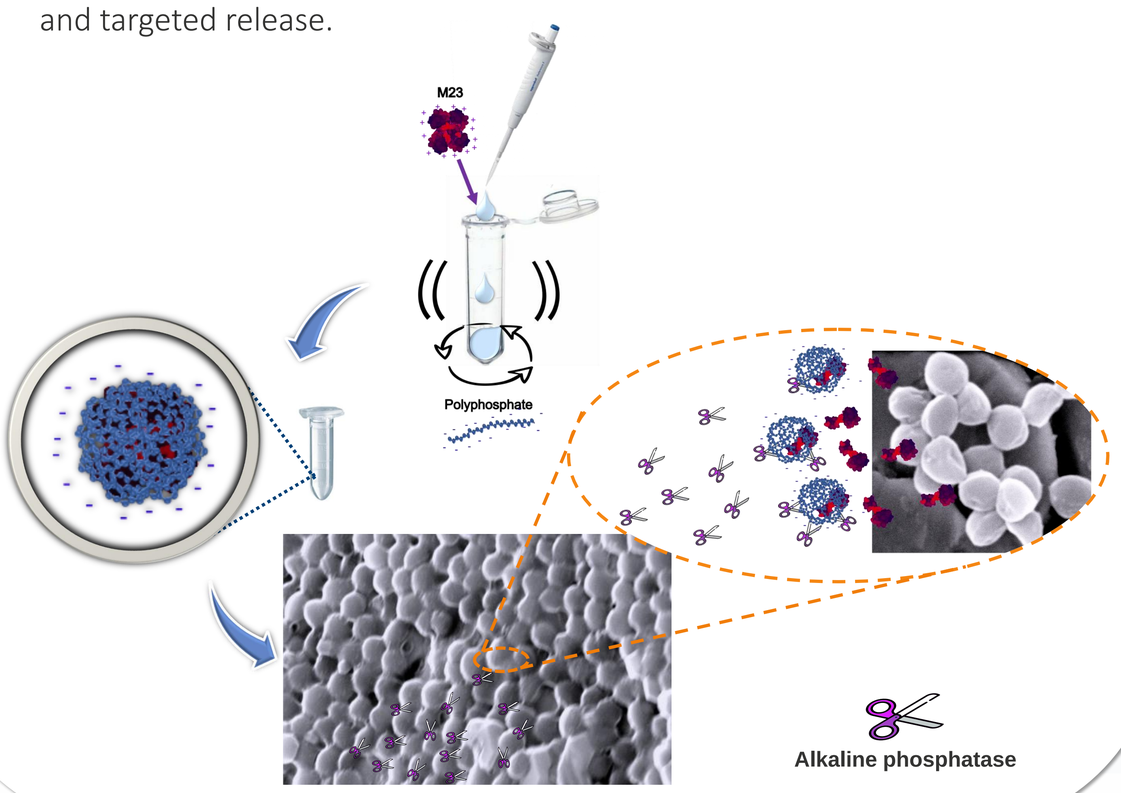
Enzyme-responsive delivery: a robust approach to enhance endolysins' efficacy against *Staphylococcus aureus* biofilm

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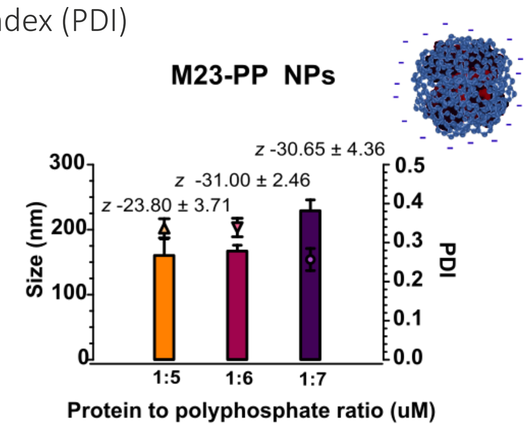
1. Introduction

- Phage endolysins efficacy to target staphylococcal biofilm is low compared to that against planktonic bacteria
- They present instability and susceptibility towards environmental stresses leading to rapid degradation
- Nanoencapsulation in enzyme-responsive platforms allow for protection and targeted release.



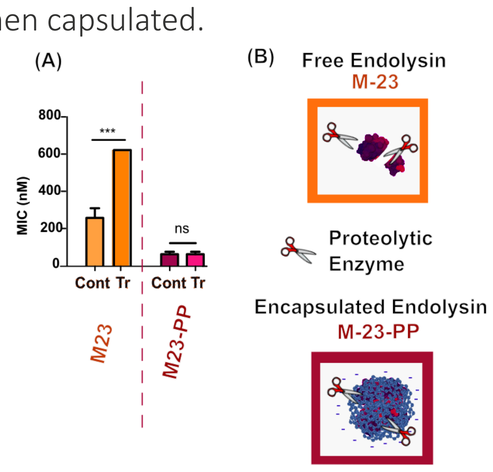
2. Nanoparticles formation and characterization

- Solution of chimeric endolysin M23 (1.25 µM) was dropped into different concentrations of polyphosphate (PP)
- A molar ratio of protein to polyphosphate of 1 to 7 led to the lowest polydispersity index (PDI)



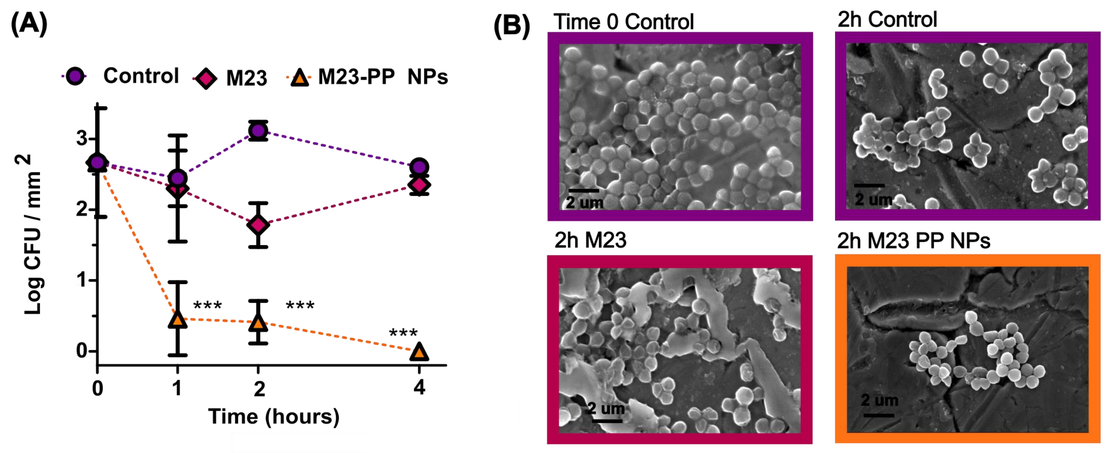
3. Protection against proteolysis

- (A) Antimicrobial efficacy was reduced upon treatment of M23 with serine protease, depicted by an increase in MIC
- For M23-PP NPs no significant changes in MIC were obtained, compared to the control
- (B) Results suggest the protection of M23 from the proteolytic effect of serine protease when capsulated.



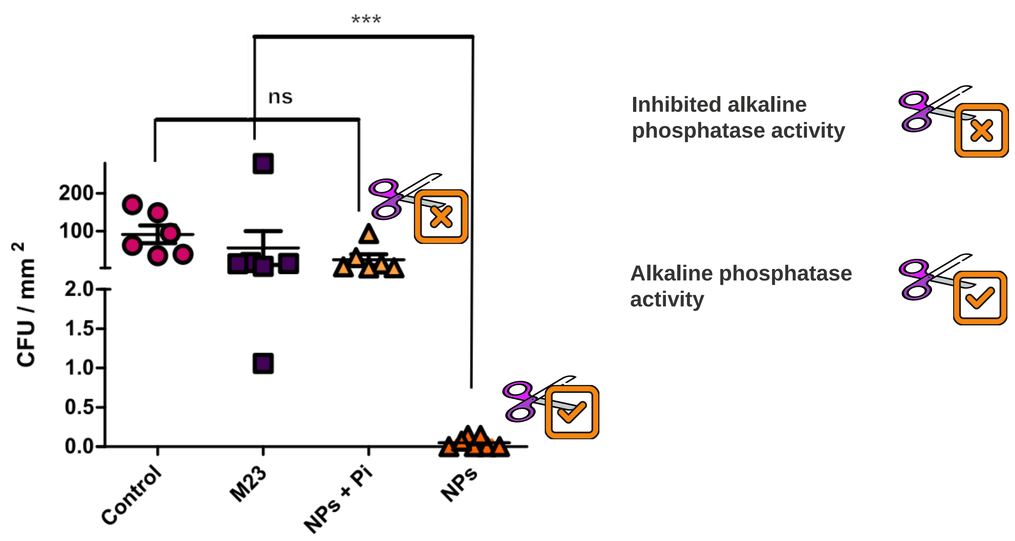
4. Time-kill assay

- (A) A pronounced increase in antimicrobial efficacy upon treatment with encapsulated endolysin was obtained compared to the free enzyme ($p<0.001$).
- (B) Cells showed damaged structure with invaginations in the cell wall after treatment with both M23 and M23-PP NPs, compared to the control.



5. Enzyme-responsive release

- M23-PP NPs killing efficacy was reduced by the presence of alkaline phosphatase inhibitor (Pi)
- Results highlight enzyme-responsive antimicrobial activity of M23-PP NPs



5. Conclusion and perspective

- A new strategy for the delivery of M23 recombinant phage endolysin was designed
- PP NPs were able to protect M23 from enzymatic degradation, while providing ALP triggered release.
- M23 PP NPs were able to efficiently eradicate *S. aureus* biofilm from an implant surface
- PP-NPs represent a promising technology for enhancing endolysins' efficacy against staphylococcal biofilm.

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