Development of pH-Responsive Peptide Systems for mRNA-Mediated Antimicrobial Peptide Delivery

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Background and Challenge

- mRNA therapeutics offer a promising strategy for treating infectious diseases, cancer, and genetic disorders through transient protein expression without genomic integration.
- Clinical application is limited by delivery challenges, including mRNA instability, poor cellular uptake, and inefficient endosomal escape.
- Current lipid nanoparticle delivery systems have limitations such as immunogenicity, limited tissue specificity, and restricted design flexibility.
- The pH responsive peptide – LAH4-L1 or its analogues – is very effective at delivering a wide range of cargoes *in vitro* including mRNA and could be used to produce therapeutics *in vivo* with PEGylation[1-5].

Research questions

- Can we use pH responsive cationic amphipathic peptides as a vector to help deliver cargo mRNA, encoding for HDPs (defensin) to achieve continuous production at the site of infection?
- How can the pH responsive peptide LAH4-L1 be modified to effectively deliver mRNA both *in vitro* and *in vivo*?
- What is the effect of PEGylation on uptake and endosomal escape? How can any effect of PEGylation be mitigated?

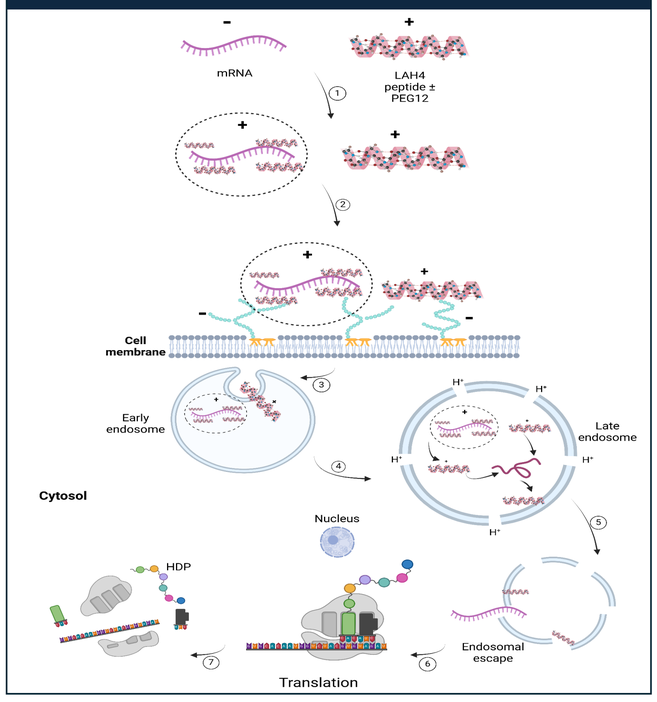
Aim and Objectives

Develop, design and characterise pH-responsive cationic amphipathic peptide vectors for efficient delivery of mRNA encoding HDPs to achieve continuous production at the site of infection.

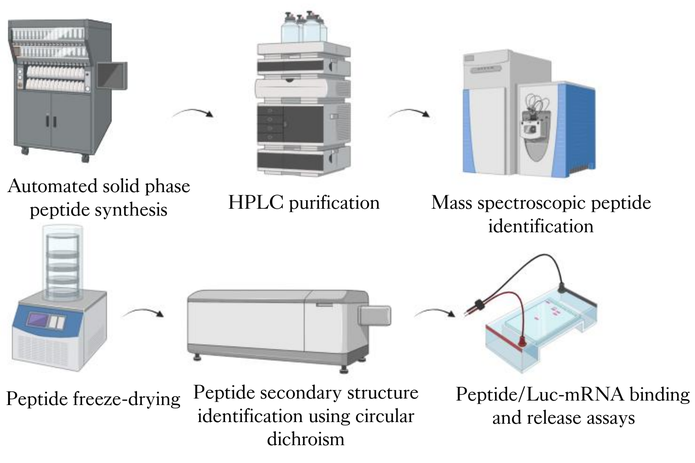
Objectives:

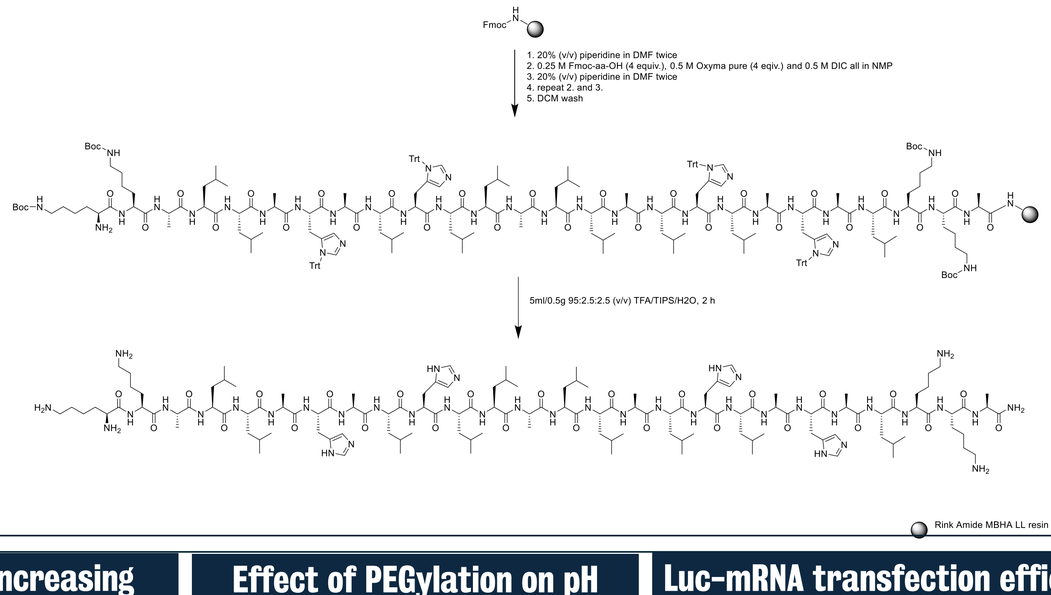
- Investigate the effect of PEGylation on the peptides' pH dependent conformational change.
- Design and modify peptide vectors to fine tune pH-triggered conformational switching.

Delivery Concept: key steps of LAH4 mediated mRNA transfection of human cells



LAH4-L1 Solid Phase Peptide Synthesis , characterisation and testing

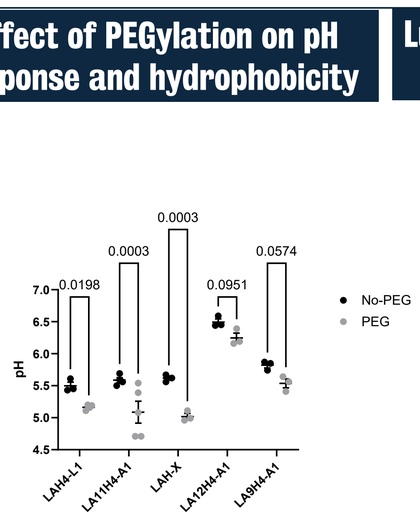


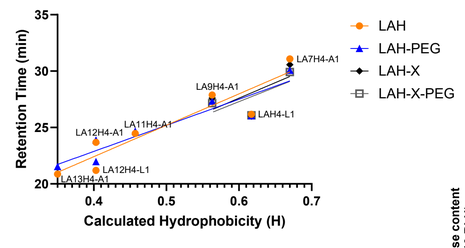




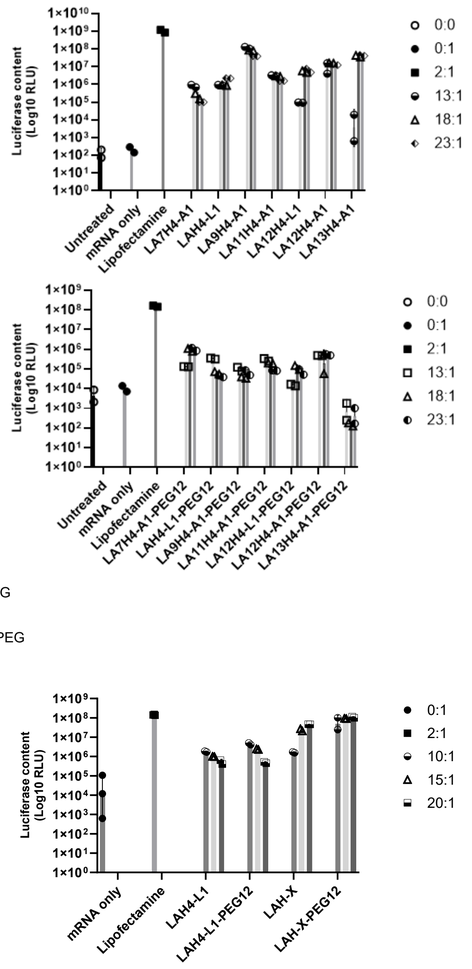
Restoring lost hydrophilic peptide α-helicity by increasing amphipathicity

Effect of PEGylation on pH response and hydrophobicity

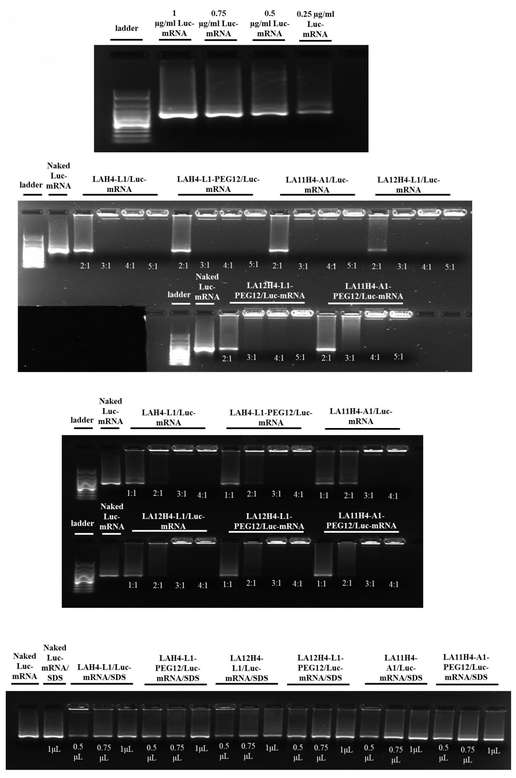




Luc-mRNA transfection efficiencies of LAH4-L1 derivatives



Luc-mRNA/peptide complexes binding and release



Conclusions

- Amphipathicity and structured partitioning of hydrophobic and hydrophilic residues within a peptide is important for folding in solution.
- PEGylation does not affect the ability of LAH4-L1 or its analogues to bind mRNA.
- Amino acid residues position within the peptide sequence affects the peptide behaviour.
- α-helicity of peptide is however poorly related to transfection efficiency.

Future Work

- Characterisation and sizing studies of mRNA/peptide nanocomplexes
- *In vitro* toxicity and characterisation of nanocomplexes.
- Selected lead pH responsive mRNA vector system will be advanced to *in vivo* pulmonary delivery studies.

References

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