Antibiotic Alternative: Silver Nanoparticles Conjugated to Virus-Like Particles

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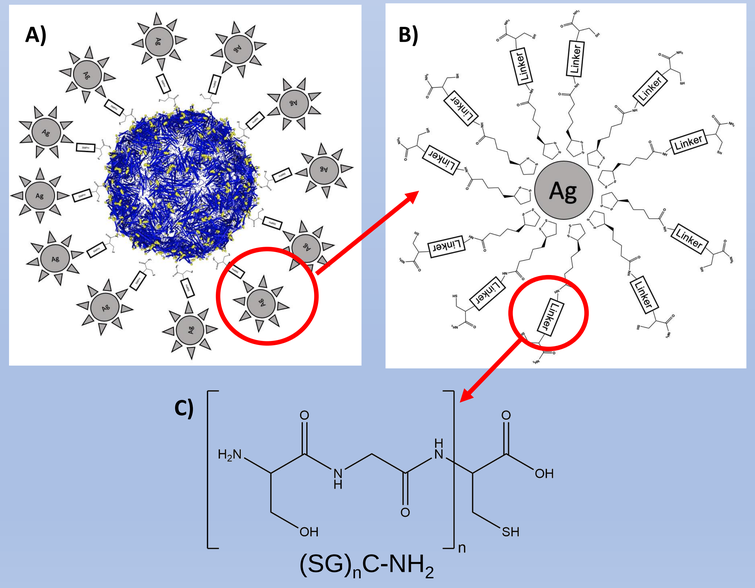
I. Background

For centuries, silver was known to have antibacterial properties. Recent studies show silver nanoparticles are potential candidates to combat multidrug-resistant bacterial infections such as those caused by *Staphylococcus aureus* and *Neisseria gonorrhoeae.*[1] Silver nanoparticles, however, face certain challenges as antibacterial agents. For example, it is difficult to balance bioactivity and biocompatibility. Larger nanoparticles elicit stronger bioactivity but can pose health issues whereas smaller nanoparticles are more biocompatible and can safely clear the body but have reduced bioactivity.[2] Meanwhile, virus-like particles (VLPs) were shown to increase bioactivities of displayed antigens.[3] Therefore, this project aims to conjugate small silver nanoparticles onto the surface of VLPs to increase the bioactivity of the nanoparticles while maintaining biocompatibility of small nanoparticles. The VLP-nanoparticle complexes will be conjugated via lipoic acid (LA) peptide derivatives. Thus far, we successfully synthesized Qbeta VLPs using standard molecular biology techniques (data not shown). Currently, we are synthesizing various LA peptide ligands of different composition, length, and flexibility using solid phase peptide synthesis (SPPS) and characterized by HPLC and MALDI-TOF.

Nanocluster VLP Complexes

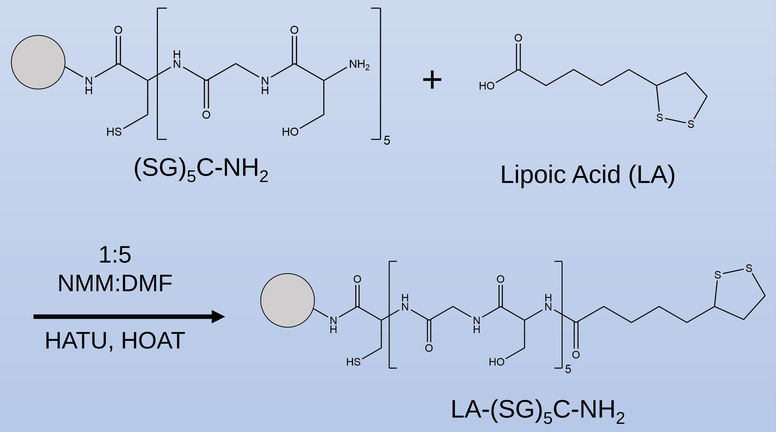


A) The silver nanoparticles will be conjugated to the lysines on the surface of the VLP via a bifunctional crosslinker (SMPH) to the cysteine of the lipoic acid peptide derivatives. B-C) Various peptide and PEG linkers will be used to conjugate the silver nanoparticles to the VLPs.

II. Procedure

1. Synthetic Peptides
   a) Standard Fmoc Solid Phase Peptide Synthesis (SPPS) on a PRELUDE® X Synthesizer with rink amide resin
   b) Deprotect N-terminal amine for LA conjugation
2. Manual conjugation of lipoic acid (LA)
   a) Added 1:1:1 molar ratio LA, HATU, and HOAT in 3mL of 1:5 NMM in DMF and stir for 30 minutes
   b) Wash with DMF and DCM
3. Characterize peptide using with ESI

Synthesis of LA-$(SG)_5$C-NH_2



List of Linkers

Linker	Sequence	Expected MW (g/mol)	Observed MW (g/mol)
LA	$LA\text{-}C\text{-}NH_2$	308.47	-
$(SG)_2$	$LA\text{-}(SG)_2C\text{-}NH_2$	597.74	596.683 = (-) H
$(SG)_3$	$LA\text{-}(SG)_3C\text{-}NH_2$	741.87	780.126 = (+) K
$(SG)_4$	$LA\text{-}(SG)_4C\text{-}NH_2$	886.00	909.059 = (+) K
$(SG)_5$	$LA\text{-}(SG)_5C\text{-}NH_2$	1030.13	1067.705 = (+) K
PEG1	$LA\text{-}(PEG)C\text{-}NH_2$	467.66	Pending
PEG2	$LA\text{-}(PEG)_2C\text{-}NH_2$	626.55	Pending
PEG3	$LA\text{-}(PEG)_3C\text{-}NH_2$	785.65	Pending

VI. Refences and Acknowledgements

1) Galdiero, S., et al., **2011**. 16(10): p. 8894-8918.
2) Lara, H.H., et al., **2019**. 4(26): p. 21914-21920.
3) Mohsen, M.O., et al., **2017**. 34: p. 123-132.

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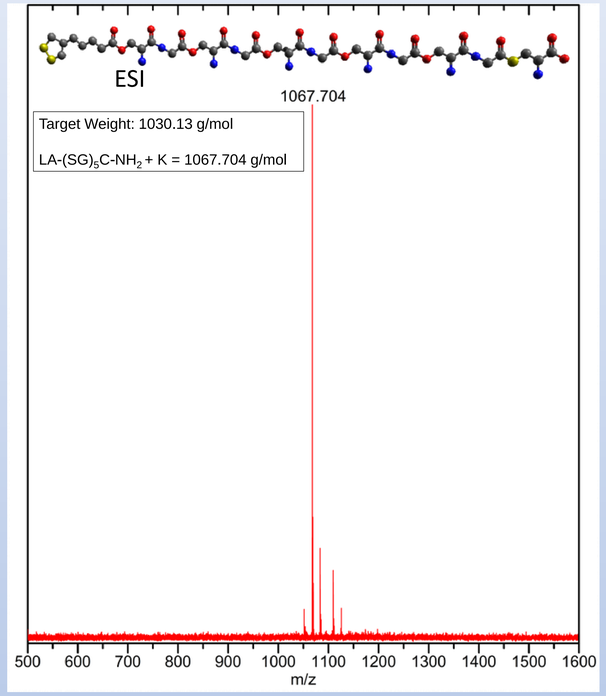
III. Results

ESI of LA-$(SG)_5$C-NH_2 Synthetic Peptide



ESI confirms successful conjugation of LA to $(SG)_5$ linker with the major peak having a mass of 1067.704 g/mol that corresponds to the addition of K (~40 g/mol).

IV. Conclusion

- $(SG)_5C\text{-}NH_2$ linker was successfully synthesized and coupled to LA
- VLPs were synthesized and characterized by lab mate

V. Future Studies

- Synthesize $LA\text{-}SG_nC\text{-}NH_2$ derivatives and characterize
- Conjugation of linkers with silver nanoparticles to form nanocluster VLP. Complexes then characterize using ESI, TEM, etc.
- Test Complexes on various biofilms