The Great Escape: Defining the Molecular Basis of Cellular Uptake and Endosomal Escape of ADCs

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Introduction

Antibody-drug conjugates (ADCs) offer a promising cancer treatment, but only **0.1% of the total dose** reaches the target [1] due to **low cellular uptake and inefficient endosomal escape**.[2,3] This project explores **the molecular basis** of ADC uptake and endosomal escape, aiming to enhance therapeutic efficacy. Incorporating **endosomal escape peptides (EEPs)** and **cell penetrating peptides (CPPs)** into ADCs could boost payload delivery [4,5,6], with the properties, safety and efficacy of novel delivery peptides and peptide-antibody conjugates requiring validation.

Aims & Objectives

This PhD aims to design and characterise peptides to promote the cellular uptake and endosomal escape of ADCs. These aims will be achieved through the synthesis of novel peptide sequences and the establishment of a robust bioanalytical pipeline for peptide and peptide-antibody conjugate characterisation.

Peptide	Sequence (N → C)	Molecular Weight (Da)
EEP	FITC-Ahx-K(N_3)ALHHALHHALHHA	2120
CPP	FITC-Ahx-K(N_3)ALAKALKALAKALKA	2138

Table 1: *Candidate peptide sequences. FITC = fluorescein isothiocyanate. Ahx = aminohexanoic acid. K(N_3) = azidolysine.*

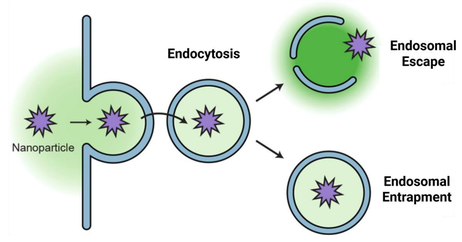

Figure 1: *Endosomal escape of nanoparticles.*

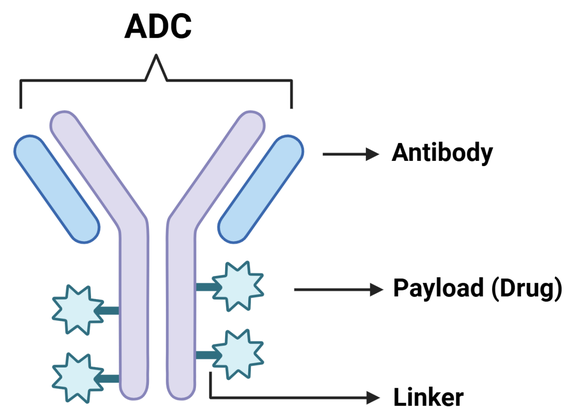

Figure 2: *Antibody-Drug Conjugate (ADC) schematic with components labelled.*

Methodology

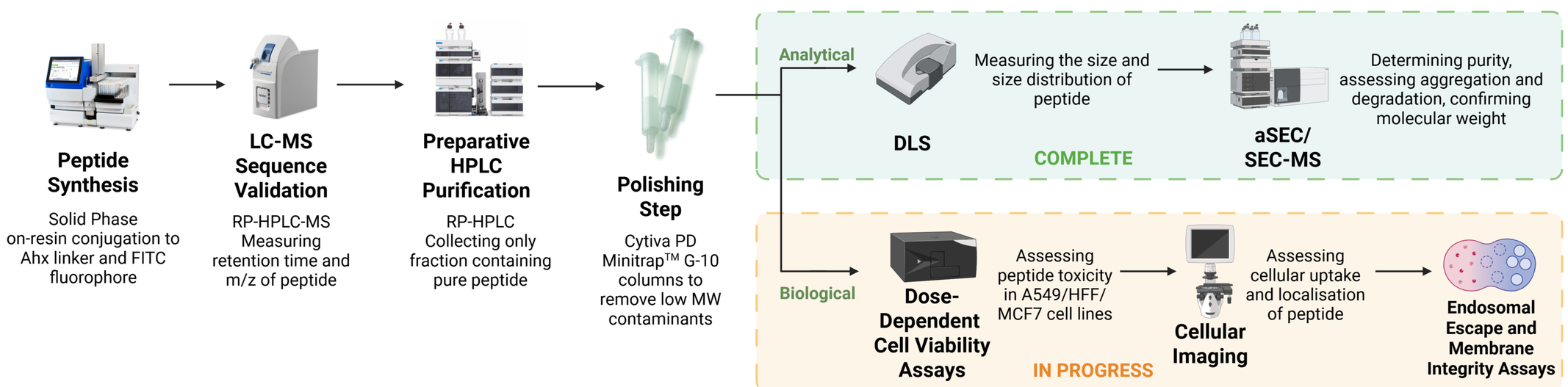

Figure 3: *Workflow for peptide synthesis, purification and analytical/biological characterisation.*

Results and Discussion

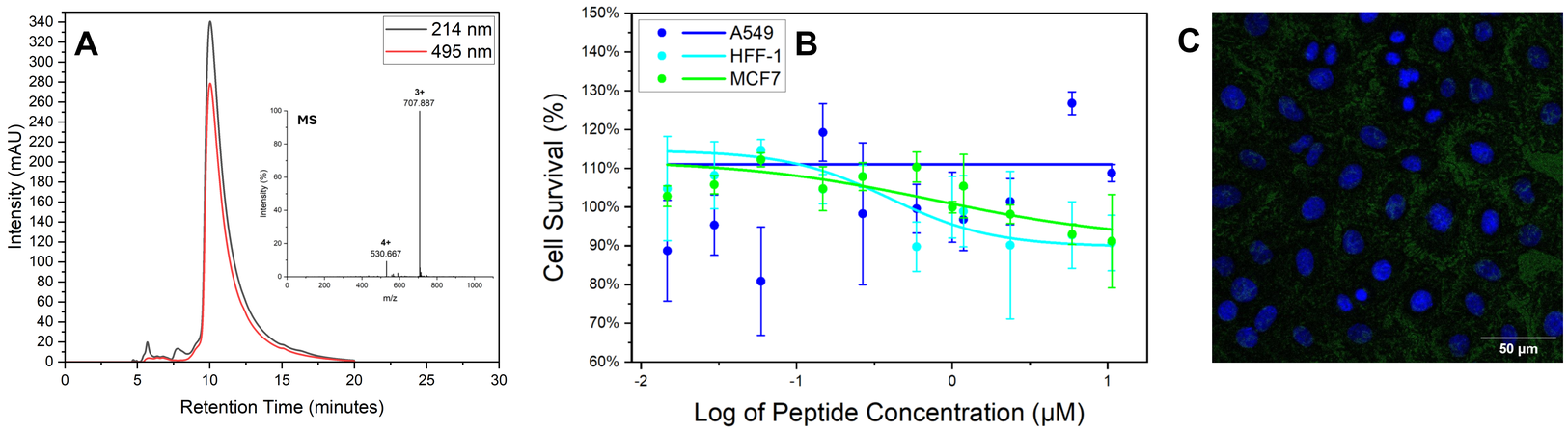

Figure 4: EEP bioanalytical characterisation results. (A) *Overlaid aSEC chromatogram profile of 5 μg EEP at 214 nm (peptide) and 495 nm (fluorophore). Deconvoluted mass spectrum (MS) inset from SEC-MS results.* ***(B)*** *CellTiter-Glo® dose-response results for 1-10 μM EEP-treated A549, HFF-1 and MCF7 cells. Error bars of standard deviation are shown.* ***(C)*** *Confocal microscopy image of EEP-treated A549 cells (10 uM, 1 hr). Blue = DAPI, green = FITC-labelled EEP.*

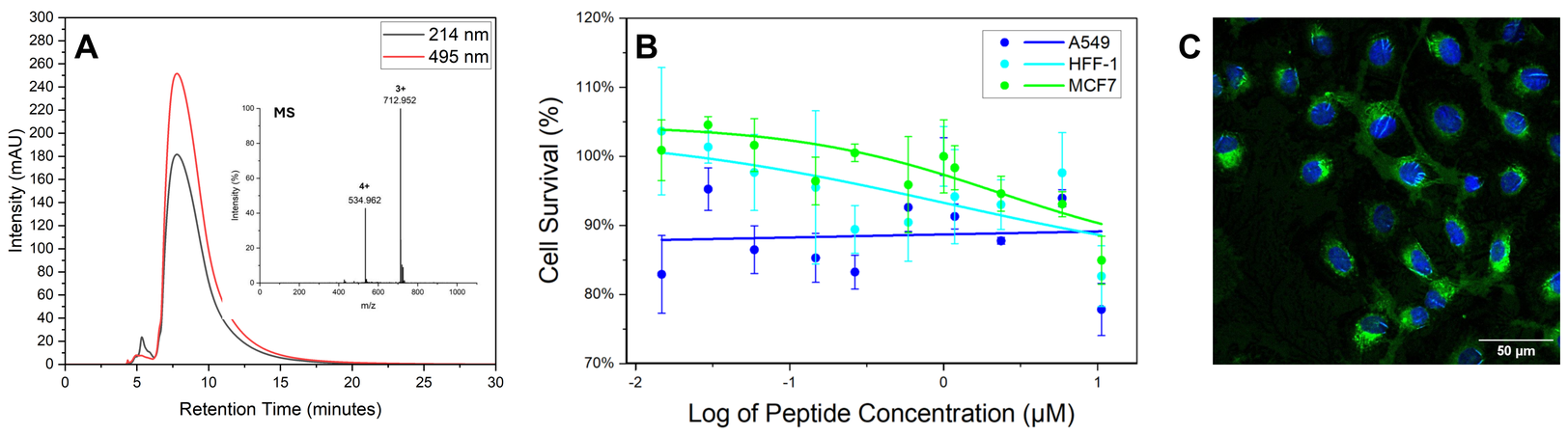

Figure 5: CPP bioanalytical characterisation results. (A) *Overlaid aSEC chromatogram profile of 15 μg CPP at 214 nm (peptide) and 495 nm (fluorophore). Deconvoluted mass spectrum (MS) inset from SEC-MS results.* ***(B)*** *CellTiter-Glo® dose-response results for 1-10 μM CPP-treated A549, HFF-1 and MCF7 cells. Error bars of standard deviation are shown.* ***(C)*** *Confocal microscopy image of CPP-treated A549 cells (10 μM, 1 hr). Blue = DAPI, green = FITC-labelled CPP.*

EEP

- Analytical SEC indicated **high purity (97%)** and low proportion of aggregate (3%)
- Expected molecular weight of **2120 Da** confirmed by mass spectrometry and LC-MS
- SEC-MS validated EEP identity and monomeric purity **(97.5%)**
- Initial dose-dependent cell viability assays in three cell lines indicate **low toxicity** of EEP
- Confocal microscopy indicated no cellular uptake of EEP, as expected due to peptide properties

CPP

- Analytical SEC with 20% acetonitrile mobile phase gave **96% monomeric purity** of CPP
- Expected molecular weight of **2138 Da** confirmed by mass spectrometry and LC-MS
- SEC-MS validated CPP identity and monomeric purity **(95%)**
- Cell viability assays showed **low toxicity** of CPP across three cell lines
- Confocal microscopy showed successful and efficient cellular uptake of CPP, validating plasma membrane activity

Conclusions & Future Work

→ The synthesised EEP and CPP were found to be **stable, pure, and non-toxic** to cells, highlighting potential as delivery agents for ADC optimisation

→ The established bioanalytical pipeline provides a **robust framework** for further peptide and ADC characterisation

Next steps will involve exploring peptide **co-dosing with trastuzumab antibody** to evaluate ADC uptake enhancement. **Endosomal escape assays** will also be developed to validate peptide function and quantify the efficiency of uptake and cytosolic delivery.

References

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