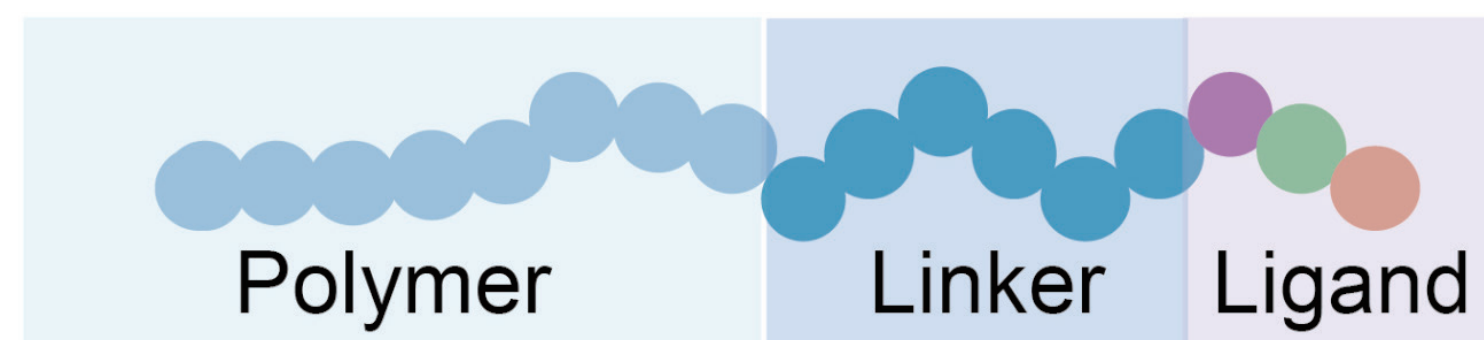


Introduction

Cationic polymers have been widely used for mRNA delivery; however, targeted delivery and endosomal escape remain major bottlenecks¹. Herein, we aimed to improve mRNA transfection efficiency by modifying the polymer with an optimised linker-ligand peptide.



Poly(β -amino ester)s (**PBAEs**) are cationic polymers widely used for mRNA delivery² and can be end-modified via Michael addition; however, this approach may promote hydrolysis of the biodegradable polymer backbone. Here, we aimed to use a rapid **photo-click**³ strategy for peptide modification and investigate its impact on polymer molecular weight. Moreover, **peptide linkers** used to conjugate the **RGD ligand** peptide to the polymer backbone, including hydrophilic (An), hydrophobic (Bn), and zwitterionic linkers (Cn), were screened to evaluate how linker design influences particle properties, cellular uptake, and mRNA transfection efficiency.

¹Huang et al. *Matter*, 2022; ²Patel et al. *Adv. Mater.*, 2019; ³Fairbanks et al. *Chem. Rev.*, 2021.

1. Photo-click chemistry enables PBAE-peptide conjugation without polymer degradation

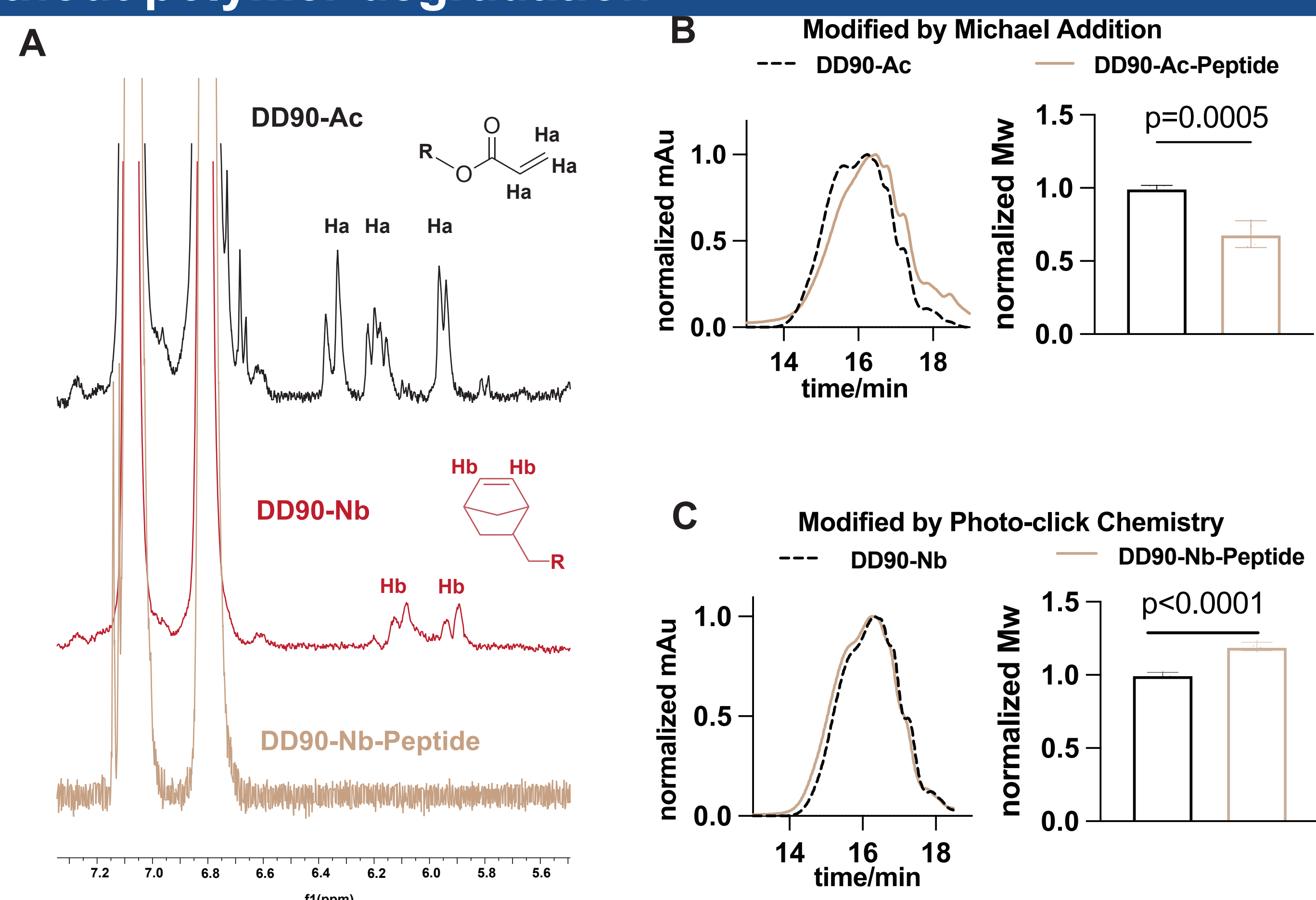


Figure 1. Peptide modification of PBAE via photo-click chemistry. (A) ¹H-NMR of DD90-Ac, DD90-Nb, and DD90-Nb-Peptide. GPC and molecular weight changes of PBAE following modification by Michael addition (B) and photo-click chemistry (C).

2. Linker screening identifies a lead linker-RGD peptide

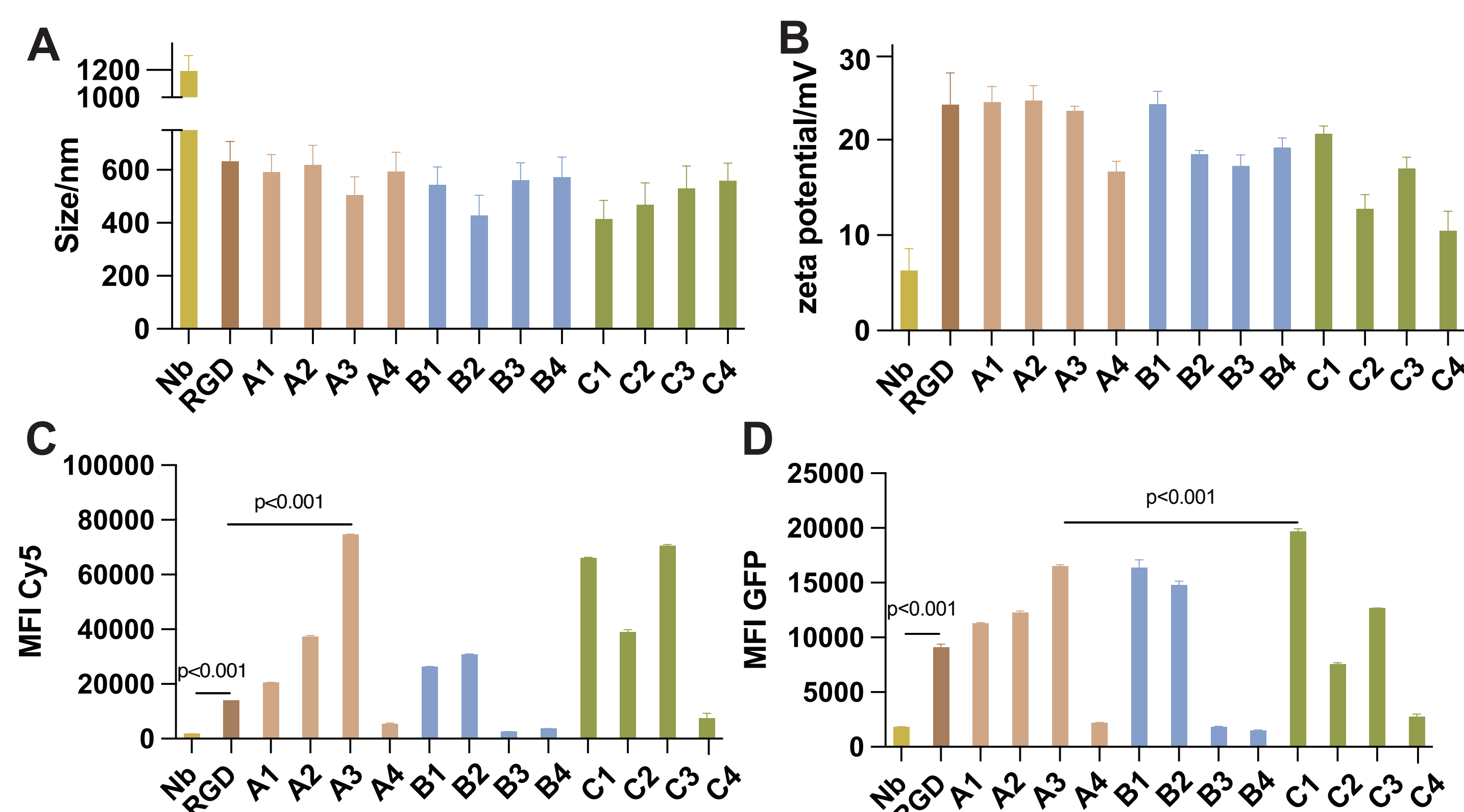
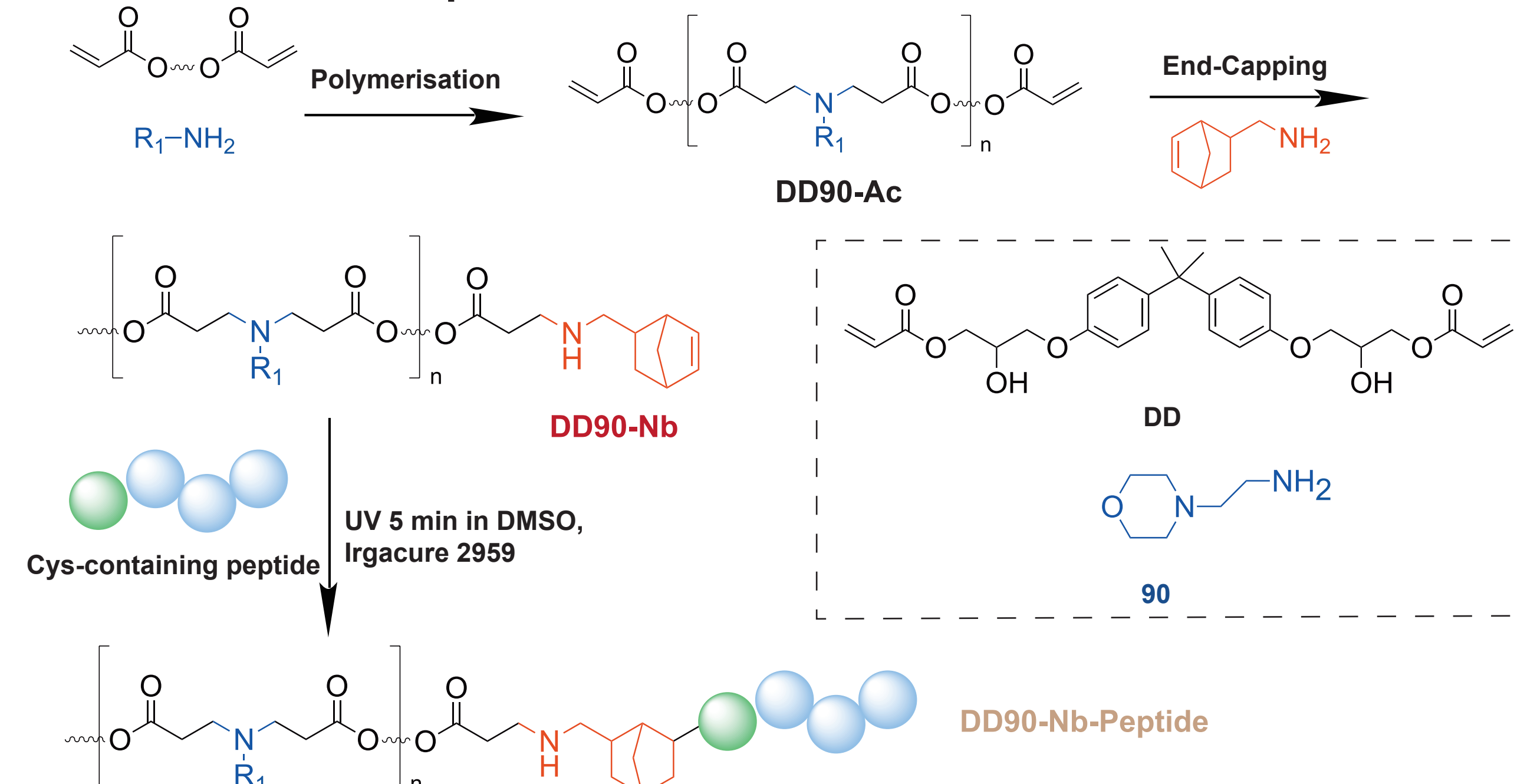


Figure 2. Screening of the DD90-Nb-Peptide library. Z-average size (A) and zeta potential (B) of polyplexes modified with different linker-RGD peptides. MFI of Cy5 (C) and GFP (D) were used to assess cellular uptake and transfection efficiency after transfection of Jurkat cells with Cy5-GFP mRNA.

Methods

Preparation of DD90-Nb-Peptide



Reaction condition: DD90-Nb and Cys-containing peptides were reacted in DMSO with Irgacure 2959 under UV irradiation for 5 mins.

Screening of the DD90-Nb-Peptide Library

- **Particle properties:** particle size and zeta potential were measured by DLS.
- **Cellular uptake and transfection efficiency:** Jurkat cells were transfected with Cy5-GFP mRNA, and cellular uptake and GFP expression were analysed by flow cytometry.

Further Validation

- Competitive binding assay and control peptide validation
- Transfection in other cell lines: 16HBE monolayers

3. Competitive binding confirms RGD-mediated uptake

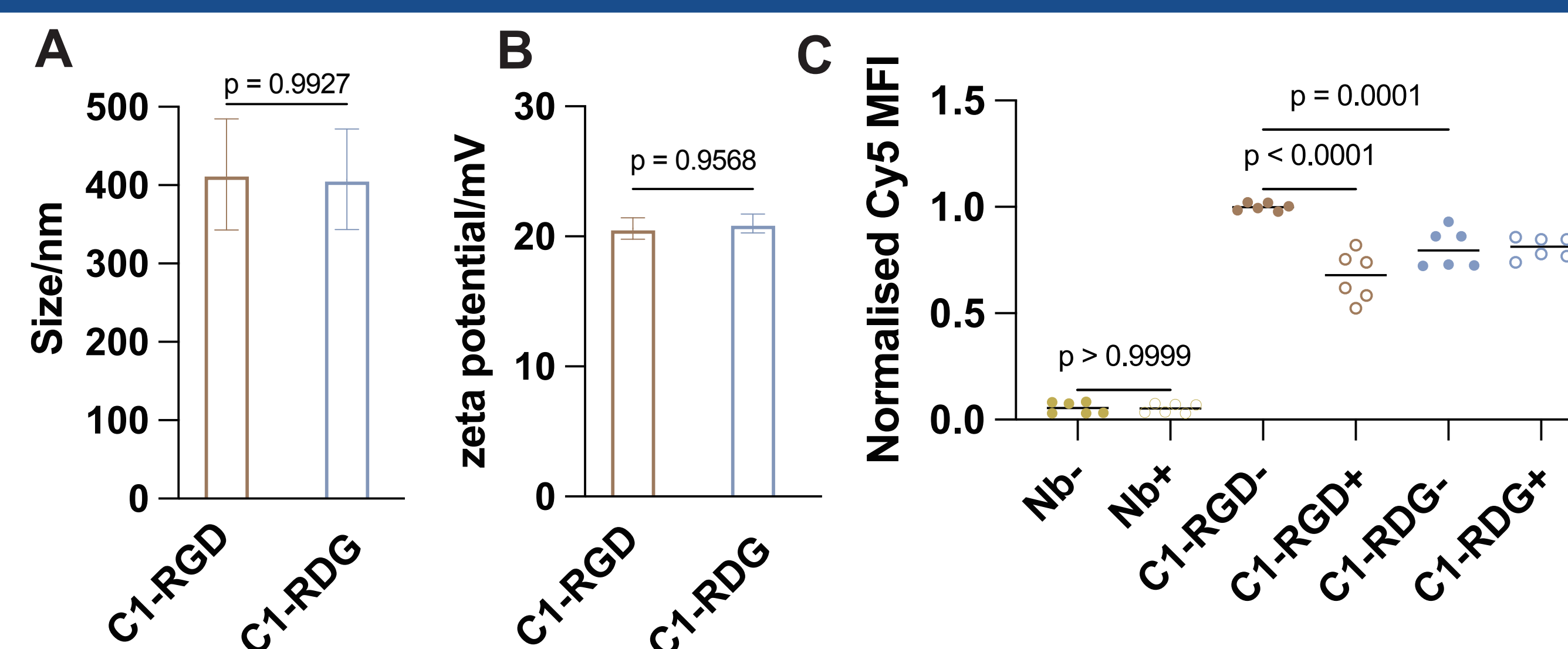


Figure 3. C1-RGD and C1-RDG polyplexes exhibited similar particle sizes (A) and zeta potential (B). (C) Cellular uptake in Jurkat cells after treatment with different polyplexes without (-) and with (+) additional free RGD peptide.

4. Lead polyplex improves transfection in 16HBE

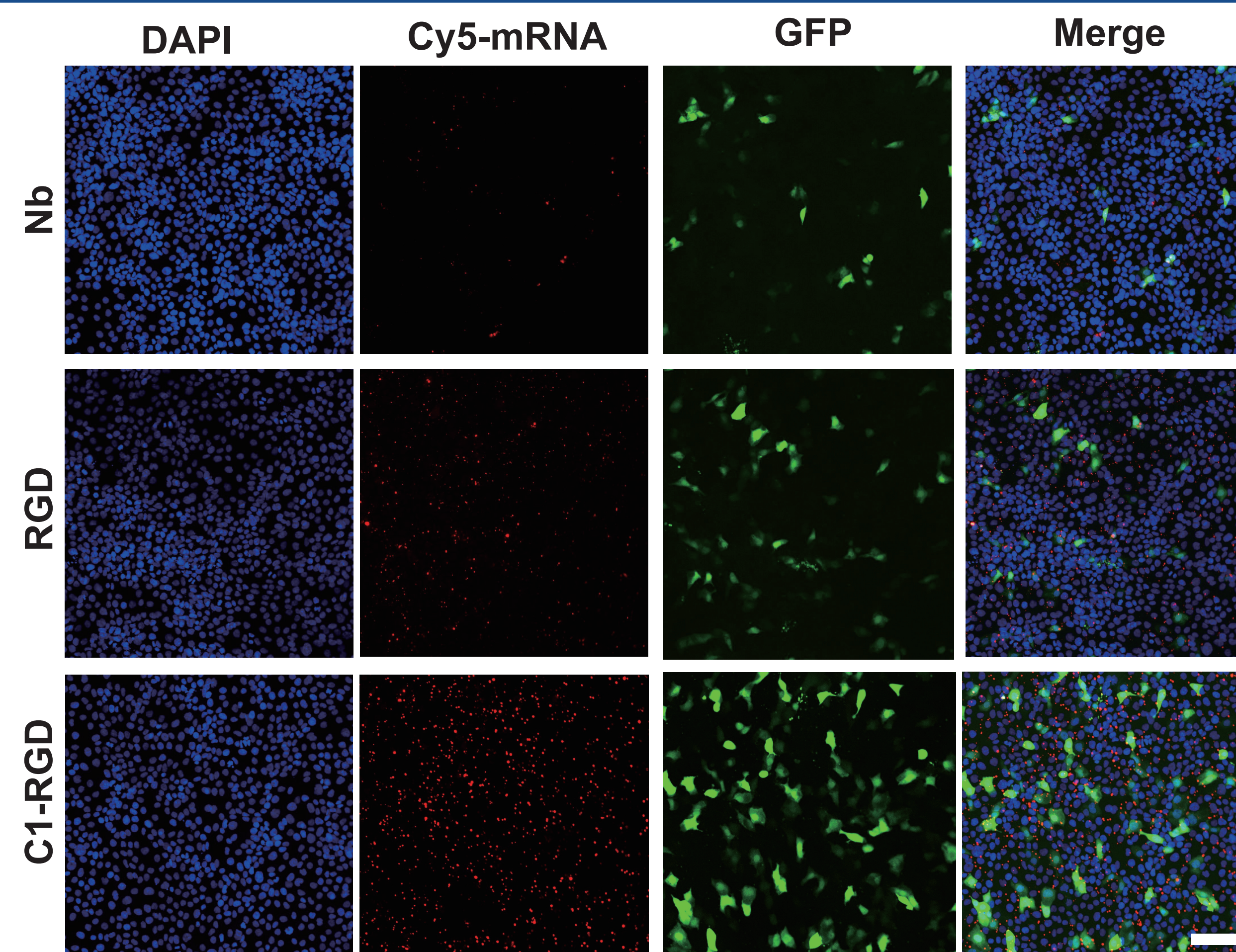


Figure 4. Confocal microscopy images of 16HBE monolayers after transfection with Cy5-GFP mRNA. Scale bar = 100 μ m.

Conclusions

- Photo-click chemistry between PBAE-Nb and Cys-containing peptides can be used to modify PBAE with peptides without causing PBAE degradation.
- Linker-RGD peptide modification can enhance cellular uptake and transfection efficiency due to the specific interaction between cells and the RGD peptide.
- Optimising the linker can improve cellular uptake and transfection efficiency.

Next Steps:

- Further optimise the formulation to reduce the particle size.
- Compare peptide-modified PBAE with conventional primary amine-terminated PBAE.
- Evaluate the delivery efficiency and safety *in vivo*.

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