

The role of nanoparticle size in intracellular transport and nuclear interactions

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1) INTRODUCTION

It is well-known that entry of nanoparticles through cell membranes and their subsequent interactions with cellular mechanisms are affected by their size and shape, amongst other factors. However, the entry and interactions of these nanoparticles beyond the cell's nuclear membrane still remains unclear. The aim of this study will be to fill in this gap in knowledge by introducing nanoparticles of different sizes into the cell nuclei and investigating their uptake and interactions with the DNA at the chromatin level.

2) NANOPARTICLE SYNTHESIS

Polymeric nanoparticles were synthesised through polymerisation-induced self-assembly (PISA) to obtain spherical particles of differing sizes.

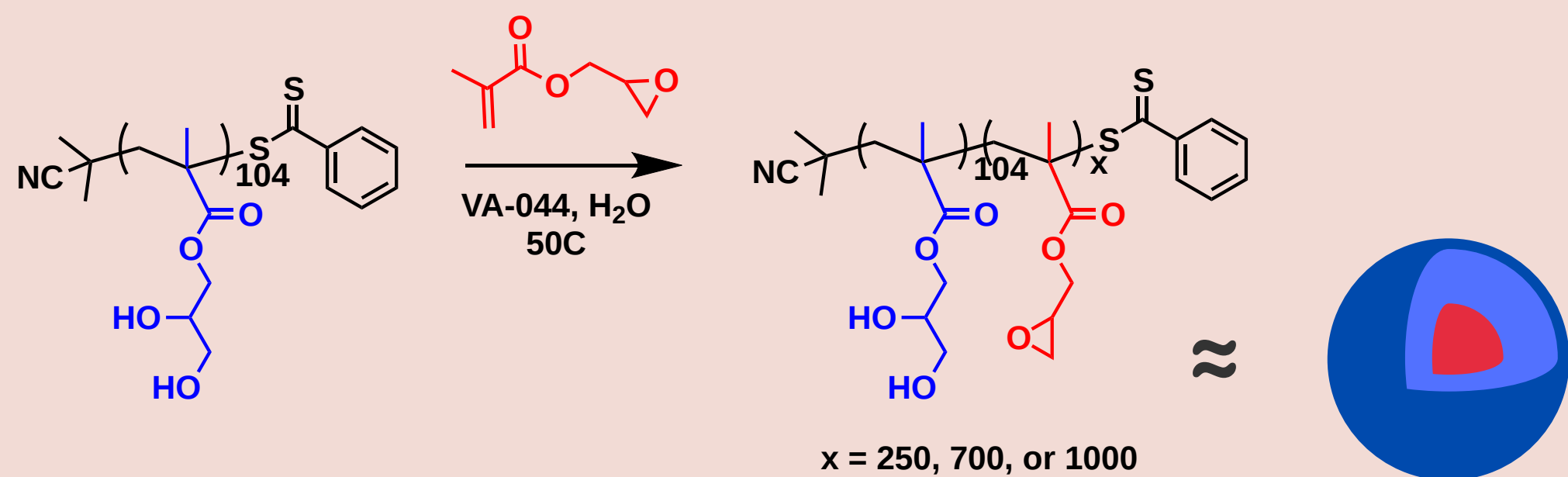


Figure 1. Chain extension of the hydrophilic poly(glycerol monomethacrylate) (PGMA) with hydrophobic glycidyl methacrylate (GlyMA) via PISA induces its self-assembly to form spherical polymer micelles. Increasing the length of the hydrophobic block results in increasing sizes of the self-assembled spheres.

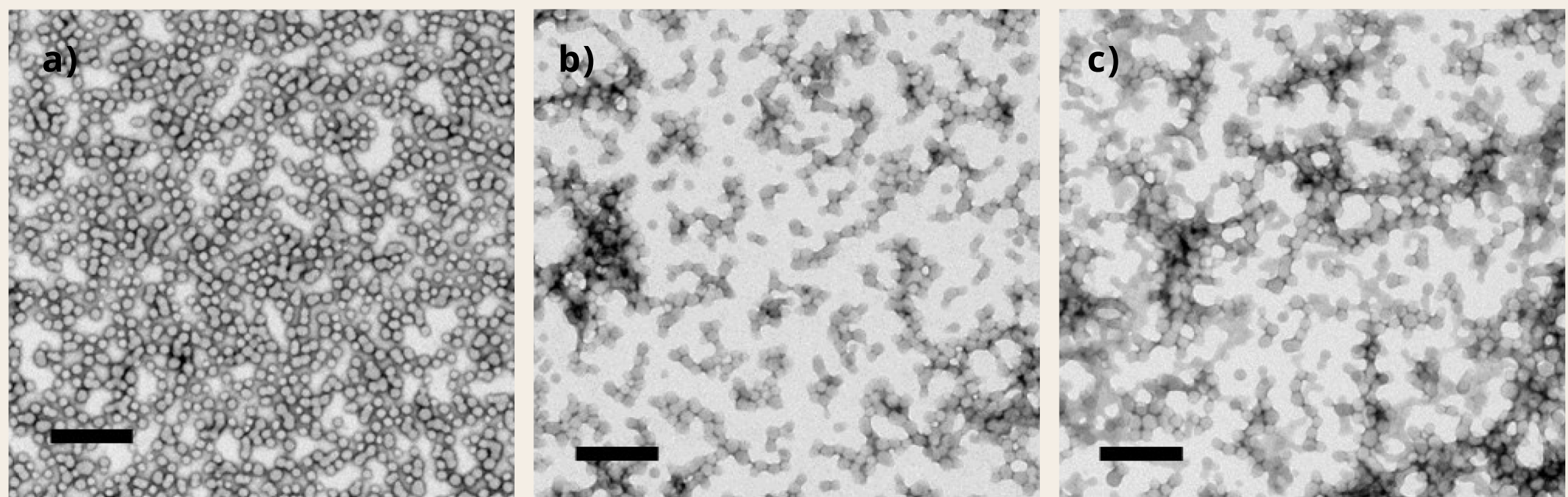


Figure 2. Representative TEM images of the PGMA-PGlyMA diblock copolymer particles prepared via PISA at 20% w/w solids in aqueous solution, where the mean degree of polymerisation for the core-forming PGlyMA block, n is equal to (a) 250, (b) 700 or (c) 1000, respectively. Black scale bars represent 200 nm in each case. Images courtesy of Armes' lab from Hatton *et al.*, Polym. Chem., 2017, 8, 4856-4868.

3) PEPTIDE CONJUGATION

The delivery of nanoparticles into the cell is not trivial and must often overcome several cellular barriers. For a nanoparticle to gain nuclear access, this can be enhanced with a nuclear localisation signal (NLS) and two NLS peptide conjugation chemistries have been investigated.

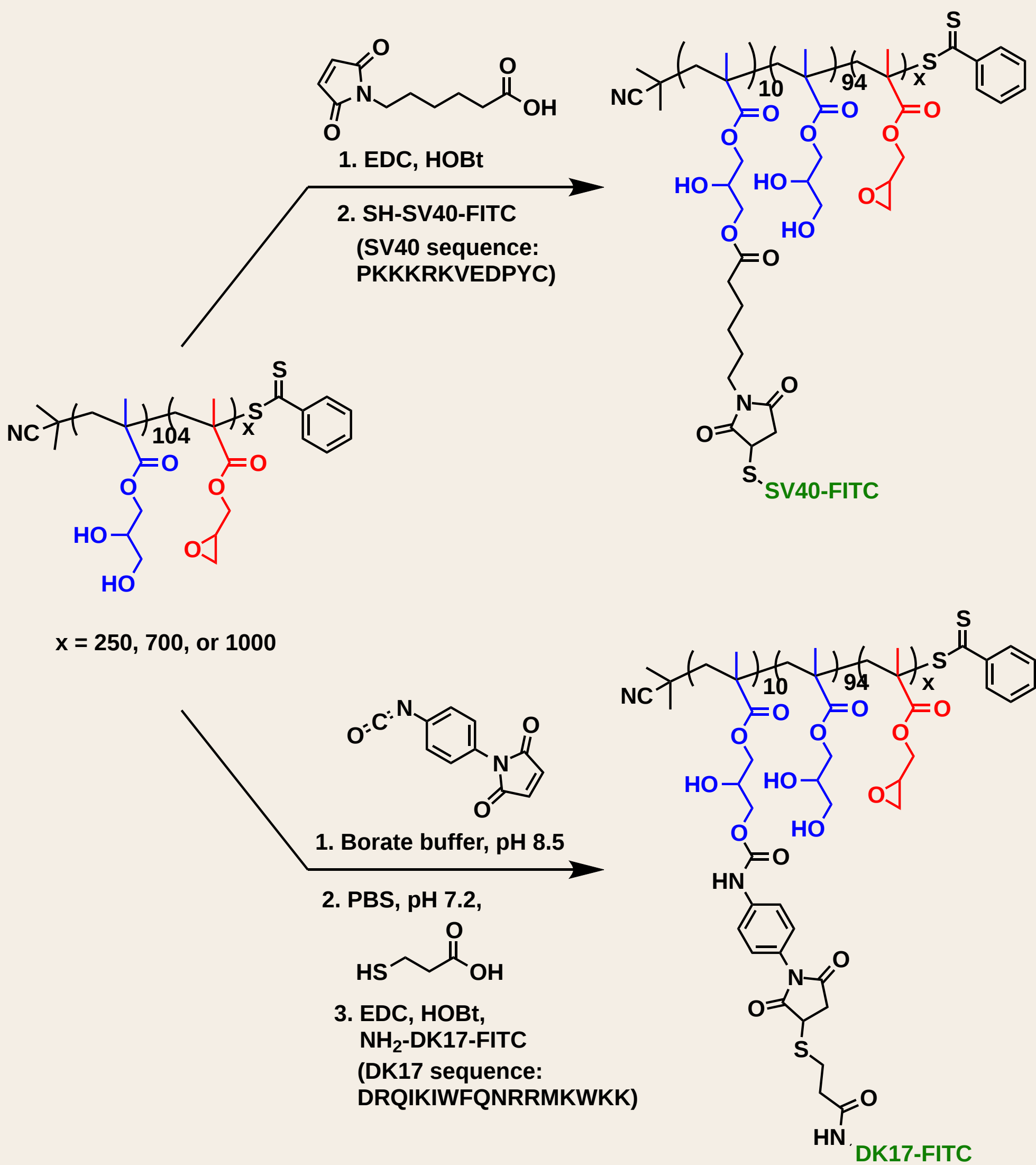


Figure 3. The hydroxyl groups of the polymer were modified to allow the conjugation of fluorescein-labelled NLS peptides. SV40 peptide was initially conjugated via cysteine residues (top route) to the particle. However, the conjugation was later optimised with attachment of the DK17 peptide via amines (bottom route) instead.

4) NANOPARTICLE CHARACTERISATION

Nanoparticles were characterised by dynamic light scattering (DLS) to obtain its average size and zeta potential. The fluorescence intensity of the nanoparticles was also confirmed.

Table 1. Average particle size (Z-avg), polydispersity index (PDI) and zeta potential of all three particle sizes before and after conjugation with SV40 NLS or DK17 NLS.

	PGMA ₁₀₄ PGlyMA ₂₅₀			PGMA ₁₀₄ PGlyMA ₇₀₀			PGMA ₁₀₄ PGlyMA ₁₀₀₀		
	Z-avg (d. nm)	PDI	Zeta (mV)	Z-avg (d. nm)	PDI	Zeta (mV)	Z-avg (d. nm)	PDI	Zeta (mV)
Before	51.76	0.07	-1.01	94.37	0.07	3.93	121.03	0.10	5.05
After +SV40	108.50	0.22	8.69	169.37	0.27	7.54	201.40	0.31	8.17
After +DK17	89.79	0.60	0.43	98.45	0.14	-0.95	122.4	0.12	-0.85

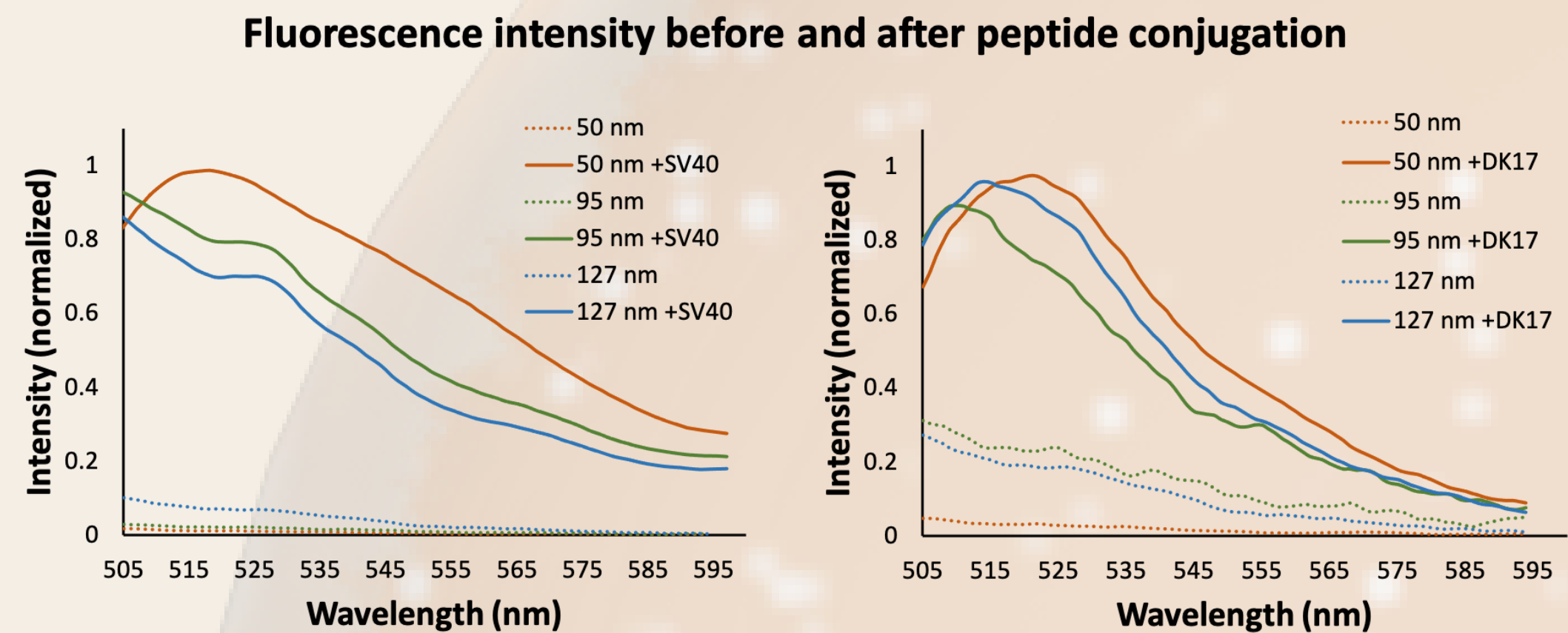


Figure 4. Fluorescence of the nanoparticles was observed after conjugating with the NLS peptide (bold line) as compared to before (dotted line) for both SV40 (left graph) and DK17 (right graph).

5) IN VITRO ASSESSMENT OF NANOPARTICLE UPTAKE INTO CELLS

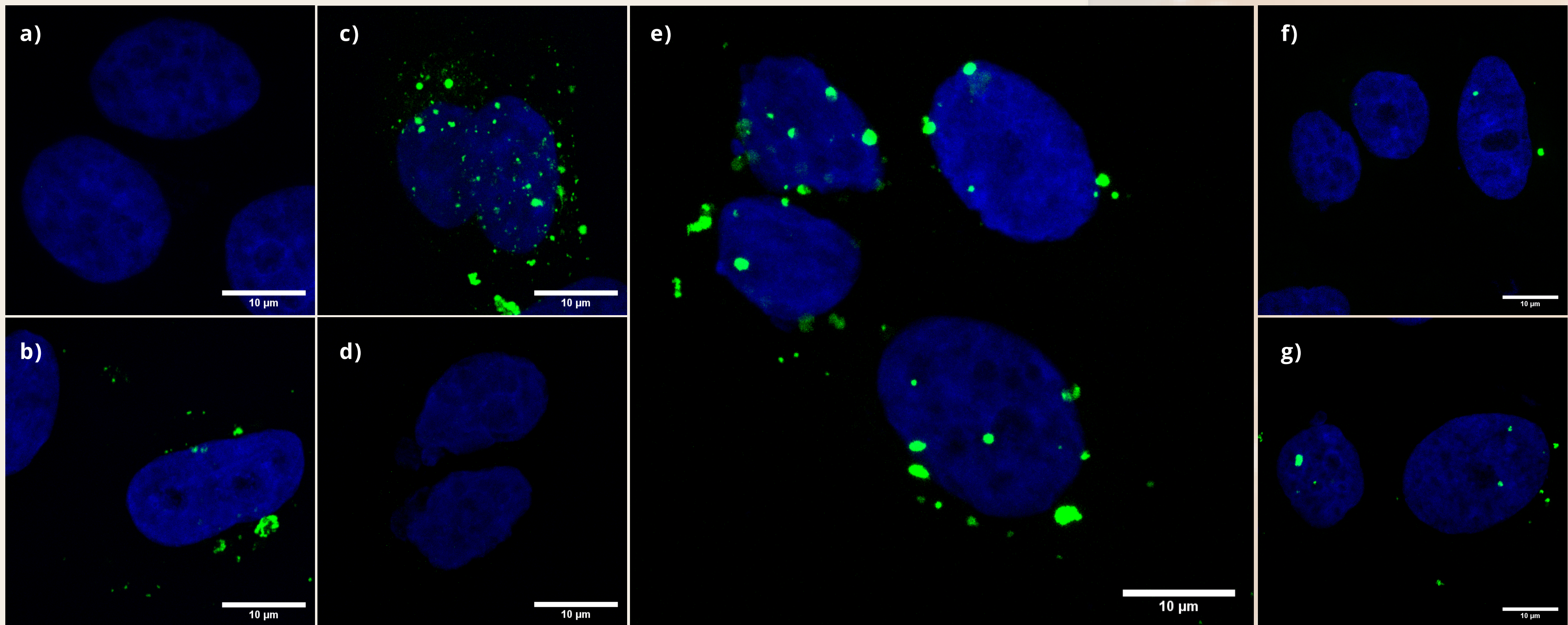


Figure 5. Confocal z-stacked images of MCF7 cells after incubation with fluorescein-labelled particles or peptide (at 0.1 mg/mL) for 4h at 37°C. a) Control cells; b) SV40 peptide-treated only; and c) DK17 peptide-treated only. For SV40-conjugated particles, no uptake was observed in the smallest particle (d) nor in the larger particles (data not shown). The optimised DK17-conjugated particles ranging in sizes from small (e), medium (f) and large (g), however, demonstrated significant uptake within the cells where the smallest particle had the most uptake within the cell nuclei. White scale bars represent 10 μm in each case. Blue (Hoechst nuclear stain); green (FITC).

6) SUMMARY & FUTURE WORK

Different sizes of polymeric nanoparticles were synthesised and modified with nuclear localisation signal. Delivery of the nanoparticle across the major cellular barrier, the nuclear envelope, was successfully demonstrated by the 50 nm nanoparticle. The interaction of the nanoparticle with the DNA at the chromatin level will be further investigated using next-generation sequencing techniques such as ATAC-sequencing.