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Tailoring the self-assembly of cyclic peptide scaffolds for prostate cancer imaging

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Introduction

Advancements in the design of peptide self-assemblies have opened the potential for nanostructuresto be used in cancer diagnosis and imaging. The use of peptide nanomaterials as imaging probes represents a promising alternative owing to its tailorable design that provides specificity towards molecular targets.[1-4] In the context of cancer diagnostics, optical imaging holds immense potential, where precise targeting of biomarkers, like the prostate-specific membrane antigen (PSMA), is crucial for cancer detection.

In our exploration of optical imaging agents for cancer, we focused on the design and co-assembly of cyclic peptide templates that generate nanotubes. These nanotubes were fabricated through the pH-triggered self-assembly of D/L-alternating cyclic octapeptides.[1] The main sequence is derived from cyclo-(D-Leu-Lys-D-Leu-Tyr)$_2$. The surface of the peptide was modified to enhance its functionality, leading to three additional peptides that were critical for building the nanoprobe. The first modification involves the attachment of polymeric chains (m-PEG) to reduce nanotube aggregation. The second peptide featured the conjugation of a fluorescent dye as a signaling component. Lastly, our targeting peptide incorporated a modified PSMA-targeting ligand for cancer imaging. All the peptides were purified by reverse-phase high-performance liquid chromatography (LC-MS) and analyzed using high-resolution mass spectrometry.

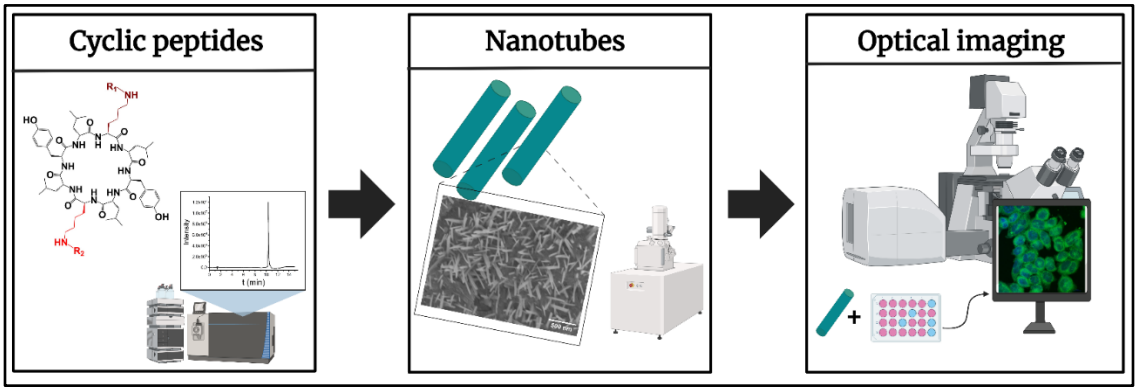

Fig. 1 General schematic representation of the research conducted

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Results and Discussion

The self-assembly behavior of all the cyclic peptides and the co-assembled systems was assessed using field emission scanning electron microscopy (FESEM). FESEM imaging revealed diameters ranging from 13 to 30 nm and lengths of 100-300 nm for most peptide systems. Notably, our targeting nanotubes, formed through the co-assembly of all four peptides, exhibited an average diameter of 28 nm and length values of 100-400 nm (Figure 2b). Circular dichroism spectroscopy revealed the presence of β-sheet-like assemblies, which were stable under room and physiological temperature conditions (Figure 2c).

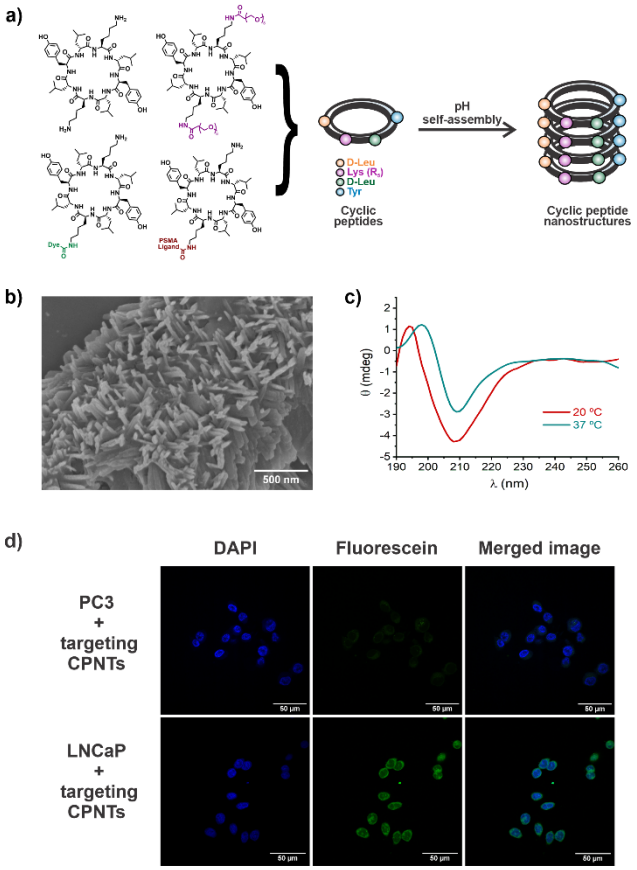

Fig. 2 (a) Schematic representation of self-assembly. (b) FESEM images of targeting nanotubes. (c) CD spectra at room and physiological temperatures. (d) Confocal fluorescence microscopy images of PC3 and LNCaP cells with targeting nanotubes after incubation for 2 h at 37 ºC.

Confocal fluorescence microscopy evaluated the targeting capabilities of the co-assembled cyclic peptides nanotubes (Figure 2d). To this end, two cancer cell lines, PC3 (PSMA-low) and LNCaP (PSMA-high) were selected. Confocal fluorescence images of LNCaP cells, treated with our targeting nanotubes, exhibited a significantly heightened fluorescence signal on cell membranes compared to non-targeting nanotubes. This confirms the interaction of our developed nanoprobes with PSMA overexpressed in LNCaP cells. Further analysis via Z-stacking indicated partial internalization of the targeting nanotubes, suggesting a potential endocytic mechanism. Confocal fluorescence images of PC3 cells, treated with our targeting nanotubes, exhibited low fluorescence signal as was expected due to the reduced presence of PSMA in this cell line.

In conclusion, our findings underscore the enhanced target specificity that can be achieved by engineering peptide nanotubes with tailorable functionalities, expanding the use of co-assembled peptide nanoprobes to the realm of molecular imaging and offering possible pathways towards clinical translation.

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