Supplementary Material (ESI) for Chemical Communications

Supplementary Information

Self-Assembly of Peptide Rod-Coil: A Polyproline Rod and a Cell-Penetrating Peptide Tat Coil

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Synthesis of supramolecular building blocks

Peptides were synthesized on Rink amide MBHA resin (Anaspec, USA) by using standard Fmoc protocols on Applied Biosystems model 433A peptide synthesizer. For coupling pyrenebutyric acid to the N-terminal part of the resin-bound peptide, pyrenebutyric acid (50 μmol), HBTU (45 μmol), an DIPEA (100 μmol) in *N*-methyl-2-pyrrolidone (NMP, 3 mL) was mixed and incubated for 10 min for carboxyl activation. The activated acid was then added to the resin-bound peptide and the mixture was reacted overnight with shaking at room temperature. The resin was then successively washed with NMP and THF, and dried *in vacuo*. The dried resin was treated with cleavage cocktail (TFA: 1,2-ethanedithiol: thioanisole; 95: 2.5: 2.5) for 3h, and was triturated with *tert*-butyl methyl ether. The peptides were purified by reverse-phase HPLC (water/acetonitrile with 0.1% TFA) on C4 column. The molecular weight was confirmed by MALDI-TOF MS. MALDI-TOF MS was performed on a Applied Biosystems Voyager-DE STR Biospectrometry Workstation using α-cyano-4-hydroxy-cinnamic acid (CHCA) as a matrix. Concentrations ($P_{10}R_3$ and P_{10}Tat) was determined spectrophotometrically in water/acetonitrile (1:1) using a molar extinction coefficient of amide bond (200 M^{-1} cm^{-1}) at 230 nm. Concentrations (pyr-$P_{10}R_3$ and pyr-P_{10}Tat) was determined spectrophotometrically in water/acetonitrile (1:1) using a molar extinction coefficient of pyrene (54,000 M^{-1} cm^{-1}) at 335 nm.

Circular Dichroism

CD spectra were obtained on a JASCO model J-810 spectropolarimeter. Scans were performed in 1 mm path length cuvette and were repeated five times at a scan rate of 100 nm/min. Molar ellipticity was calculated per amino acid residue.

Dynamic Light Scattering

Dynamic light scattering experiment was performed at room temperature with ALV/CGS-3 Compact Goniometer System equipped with He-Ne laser operating at 632.8 nm. The scattering angle was 90°. Before measurement, the sample was centrifuged at 16,110 × g for 20 min to sediment any dust particles. The size distribution was determined by using a constrained regularization method.

Transmission electron microscopy (TEM)

For TEM experiment, 3 μL of an aqueous solution of sample was placed onto a holey carbon-coated copper grid, and 3 μL of 2 % (w/w) ruthenium tetroxide solution was added for positive staining. The sample was deposited for 1 min, and excess solution was wicked off by filter paper. The dried specimen was observed with a JEOL-JEM 2010 instrument operating at 120 kV. The data were analyzed with DigitalMicrograph software.

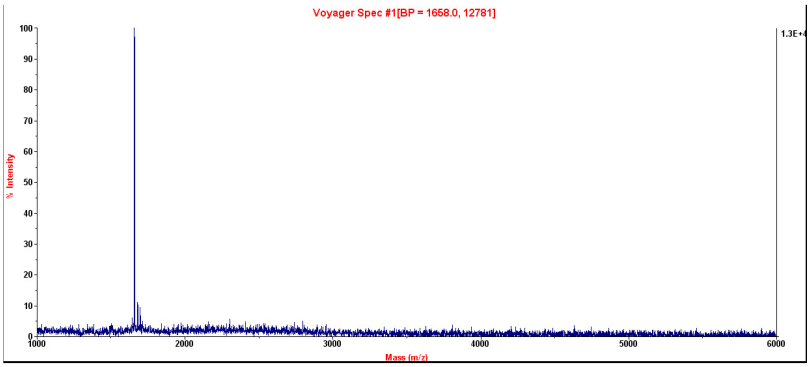
Encapsulation Experiment

To a solution of the peptide building block (20 μL, 700 μM in water) was added Rhodamine B (20 μL, 1 mg/mL in water) and the solution was sonicated for 5 min. The dye-loaded vesicles were separated from the free dye by using gel filtration chromatography over Sephadex G-50 resin (GE Healthcare). Eluent was 150 mM NaCl solution.

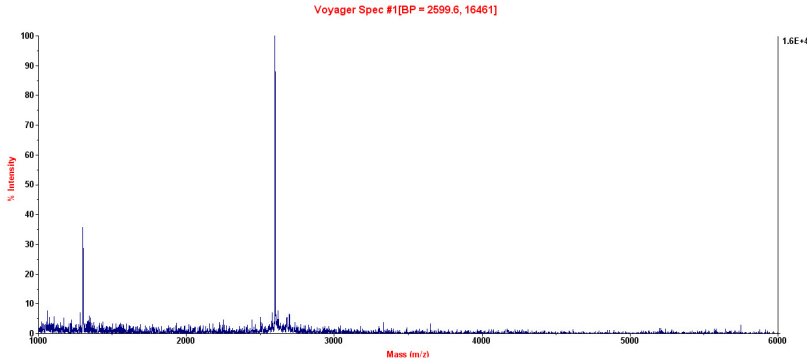
Intracellular delivery experiment

HeLa cells were seeded on sterile 12 mm-diameter coverslip in 35 mm dishes (5×10^4 cells per glass), and incubated for 24h at 37°C. The cells on the coverslip were washed 3 times with PBS and incubated at 37°C with the dye-loaded vesicles in DMEM for 3h. The final concentration of the dye-loaded vesicle was 2 μM. After PBS washing, the coverslip was inverted and placed onto glass slides over the fluorescent mounting medium (Dako, Carpinteria, CA). The cells were visualized under a confocal microscope (LSM 510 META, Carl Zeiss, Germany) with the laser exciting the sample at 405 nm or 543 nm.

a)


Voyager Spec #1[BP = 1658.0, 12781]
% Intensity
Mass (m/z)

b)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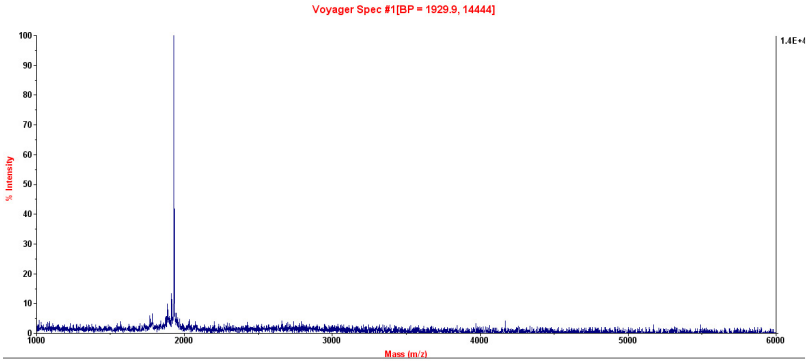
c)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Mass (m/z)

d)

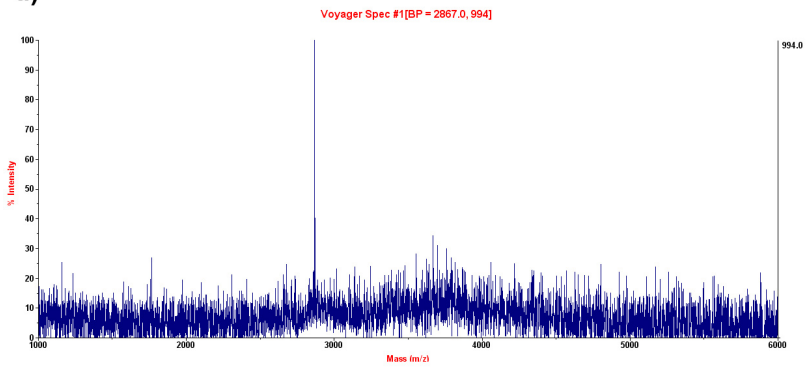
Voyager Spec #1[BP = 2867.0, 994]
994.0
% Intensity
Mass (m/z)

Figure S1. MALDI-TOF MS spectrum of a) $P_{10}R_3$, b) P_{10}Tat, c) pyr-$P_{10}R_3$, and d) pyr-P_{10}Tat.

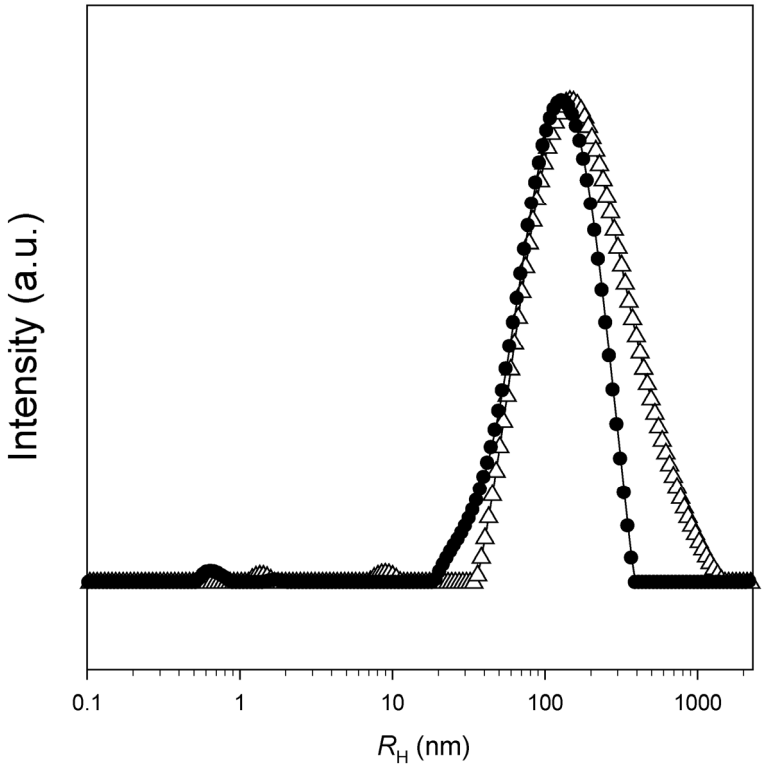

Figure S2. Distribution of R_H of pyr-$P_{10}R_3$ aggregates (closed circle) and pyr-P_{10}Tat aggregates (open triangle) in water.

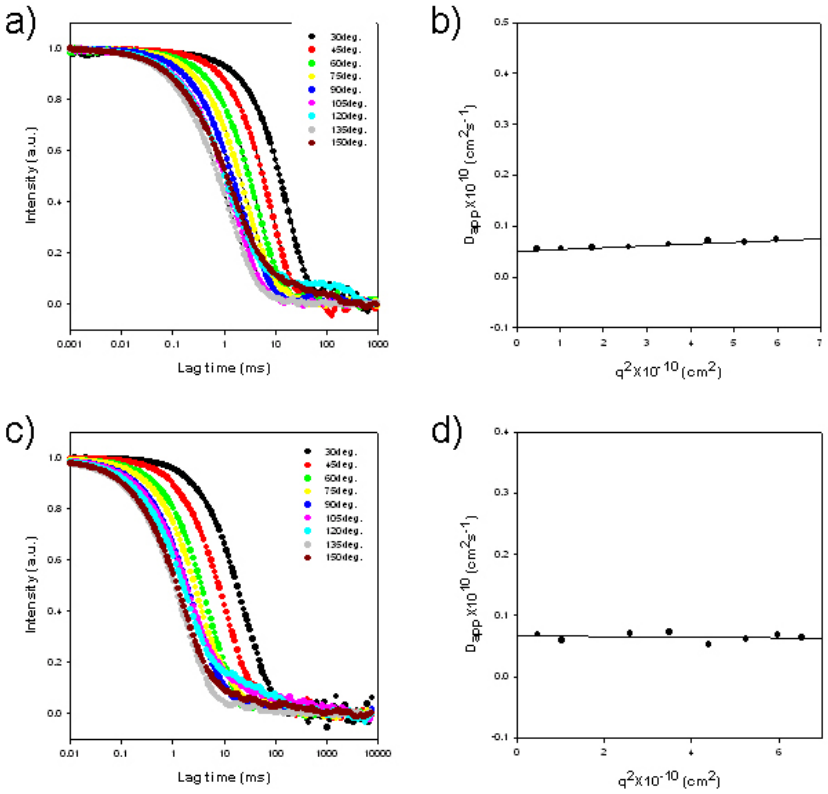

Figure S3. a) Autocorrelation functions of pyr-$P_{10}R_3$ at scattering angle from 30° to 150°. b) Angular dependence of the apparent diffusion coefficient (D_{app}) of pyr-$P_{10}R_3$ aggregates. The slope was 0.0035, consistent with the value predicted for spheres.[1] c) Autocorrelation functions of pyr-P_{10}Tat at scattering angle from 30° to 150°. d) Angular dependence of the apparent diffusion coefficient (D_{app}) of pyr-P_{10}Tat aggregates. The slope was -0.0005, consistent with the value predicted for spheres.[1]

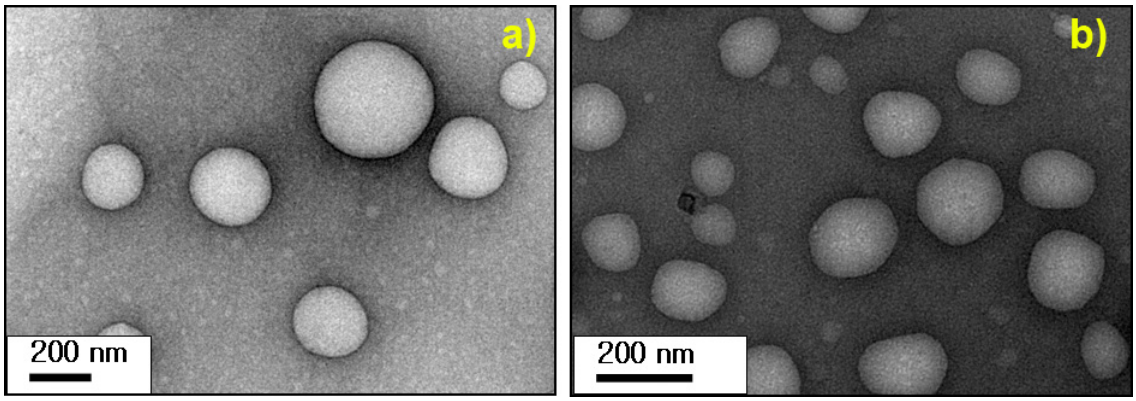

Figure S4. TEM images of a) $P_{10}R_3$ and b) pyr-$P_{10}R_3$ aggregates in water. The samples were negatively stained with uranyl acetate.

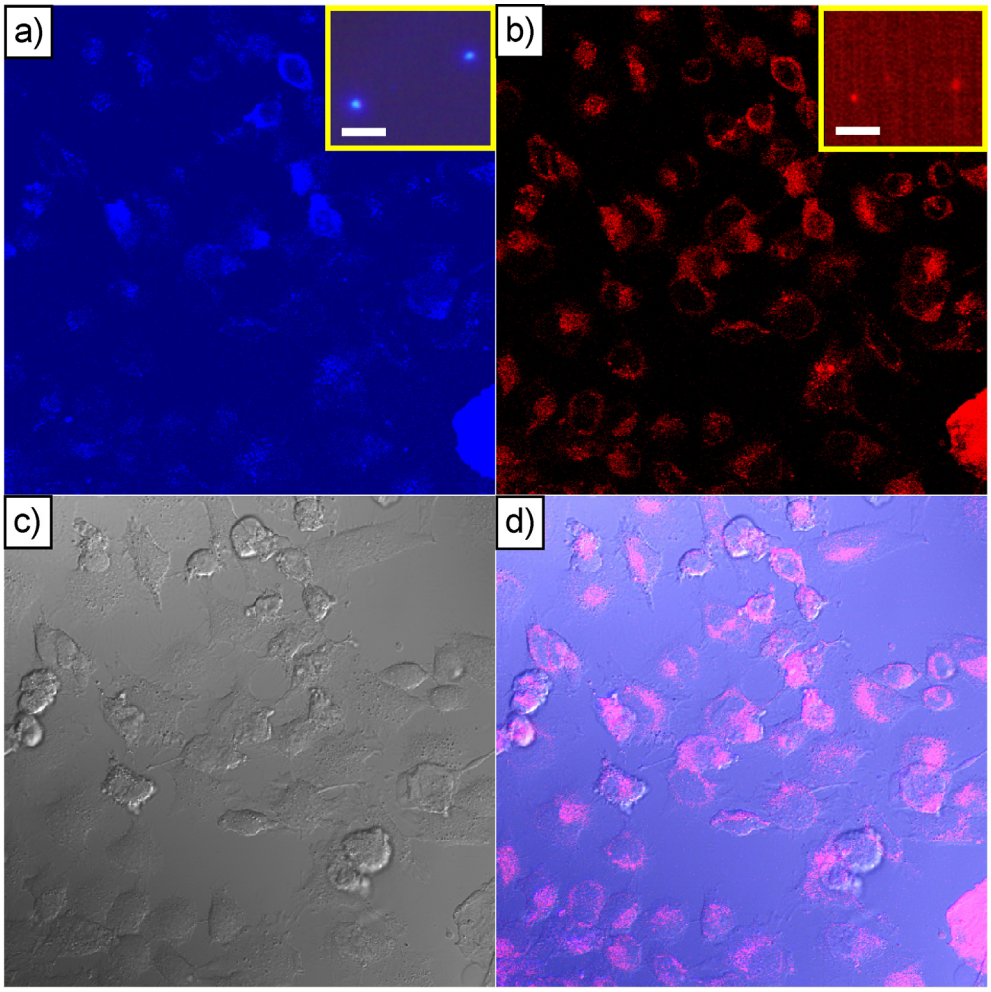

Figure S5. Intracellular delivery of hydrophilic dye-loaded pyr-P_{10}Tat vesicles. CLSM image of HeLa cells after treating the cells with Rhodamine B-loaded pyr-P_{10}Tat vesicles for 3h. a) Intracellular fluorescence from pyr-P_{10}Tat. Insets: Blue fluorescence of pyrene from Rhodamine B-loaded pyr-P_{10}Tat vesicles. Bar: 5 μm b) Intracellular fluorescence from rhodamine B. Insets: Red fluorescence of rhodamine B from Rhodamine B-loaded pyr-P_{10}Tat vesicles. Bar: 5 μm. c) Bright field image of the cells. d) Overlay of the blue and the red fluorescence images. The objects in the insets, although they are the same objects, are not in the same location between the figures because of their continuous Brownian motion.

References

1. B.-S. Kim, D.-J. Hong, J. Bae and M. Lee, *J. Am. Chem. Soc*., 2005, **127**, 16333.