Efficient and facile formation of two-component nanoparticles via aromatic moiety directed self-assembly

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Supplementary Material

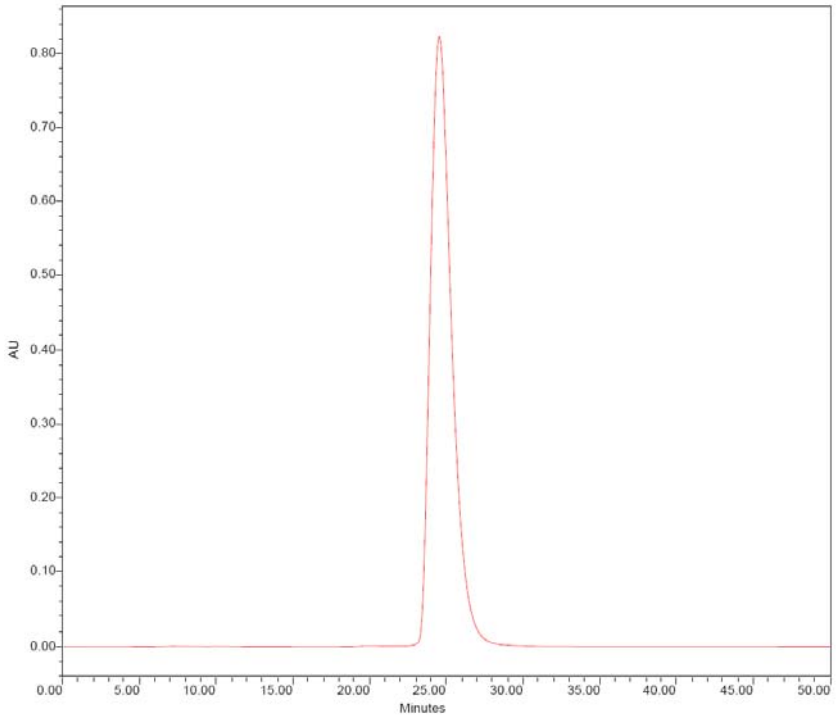
Materials

Fmoc-phenylalanine, Fmoc-Osu, Tyr(tBu)-OH and N-acetyl-phenylalanine were supplied by GL Biochem (Shanghai, China). 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) and all cell culture reagents were bought from Invitrogen. N-Hydroxysuccinimide (NHS), N,N'-dicyclohexylcarbodiimide (DCC), and other chemicals were purchased from Sigma-Aldrich unless otherwise stated. Mouse embryonic fibroblast cell line NIH/3T3 was kindly provided by Prof. Zhenguo Wu (The Hong Kong University of Science and Technology). Water was used from Thermo Scientific Barnstead Nanopure ultrapure water purification system.

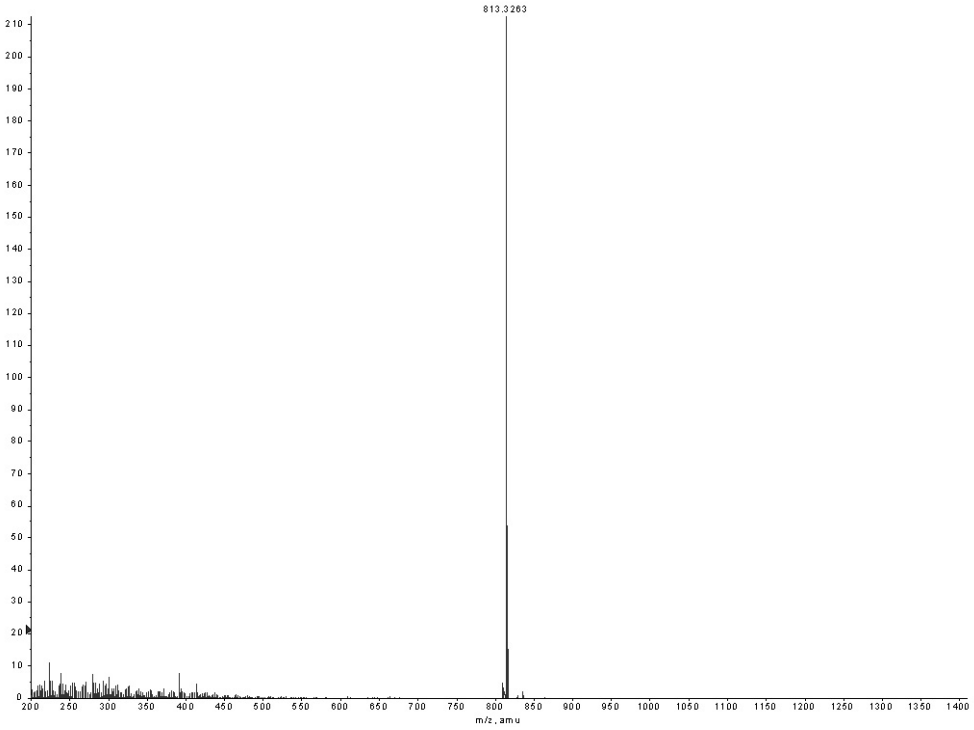
Synthesis and characterization of FTAEA

Fmoc-Osu (337.3 mg, 1 mmol) was dissolved in 100 mL of acetone. Tris(2-aminoethyl)amine (TAEA) (45 μL, 0.9 mmol) was then added into the above solution drop by drop with stirring. The reaction was left to continue at room temperature overnight. The solvent was evaporated to around 6 mL by blowing with compressed nitrogen, and then 100 mL of water was added with stirring. The precipitates were collected after centrifuging at 15000 rpm for 15 min and purified by reversed-phase high-performance liquid chromatography (RP-HPLC) (Vydac C-18, 250×10 mm column) eluting with CH_3CN/water containing 0.1% TFA (50/50 for 17 min and then from 50/50 to 60/40 over 15 min at 5 ml/min). The purified sample was characterized by RP-HPLC on an analytical column with the detection wavelength of 265 nm (purity > 98%) and by matrix assisted laser desorption ionisation time-of-flight mass spectrometry (MALDI-TOF-MS) ($[M + H]^+$ calculated = 813.96, observed = 813.33).

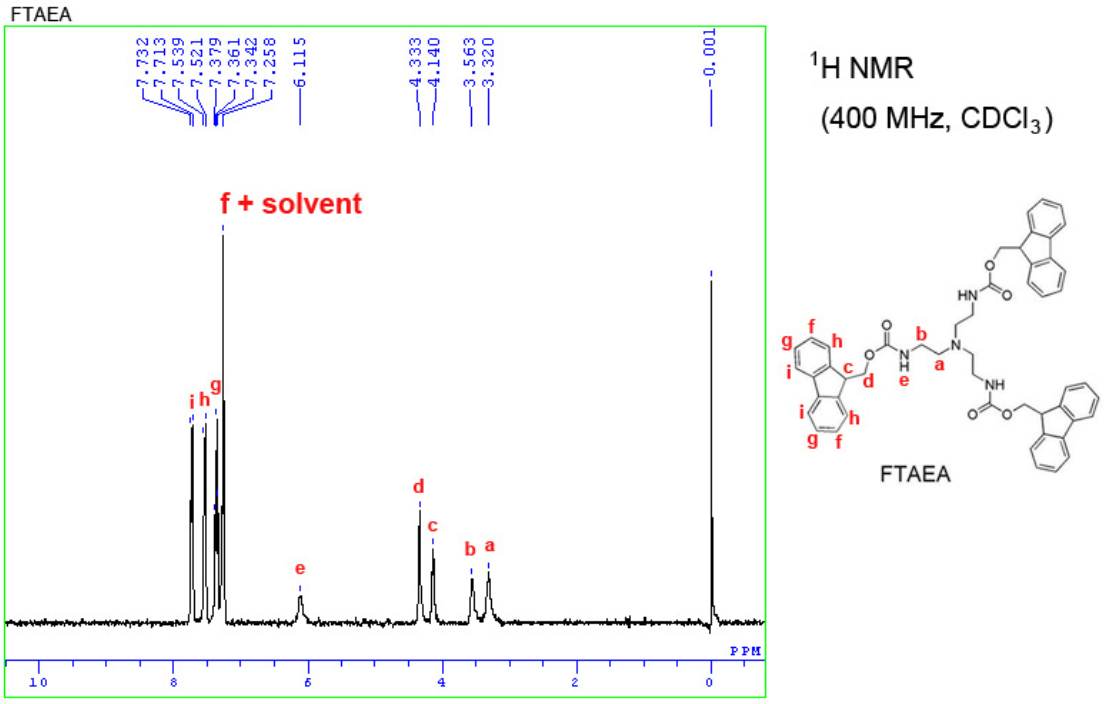
HPLC analysis of purified FTAEA:



MS result of purified FTAEA:



^{1}H NMR spectra of purified FTAEA:

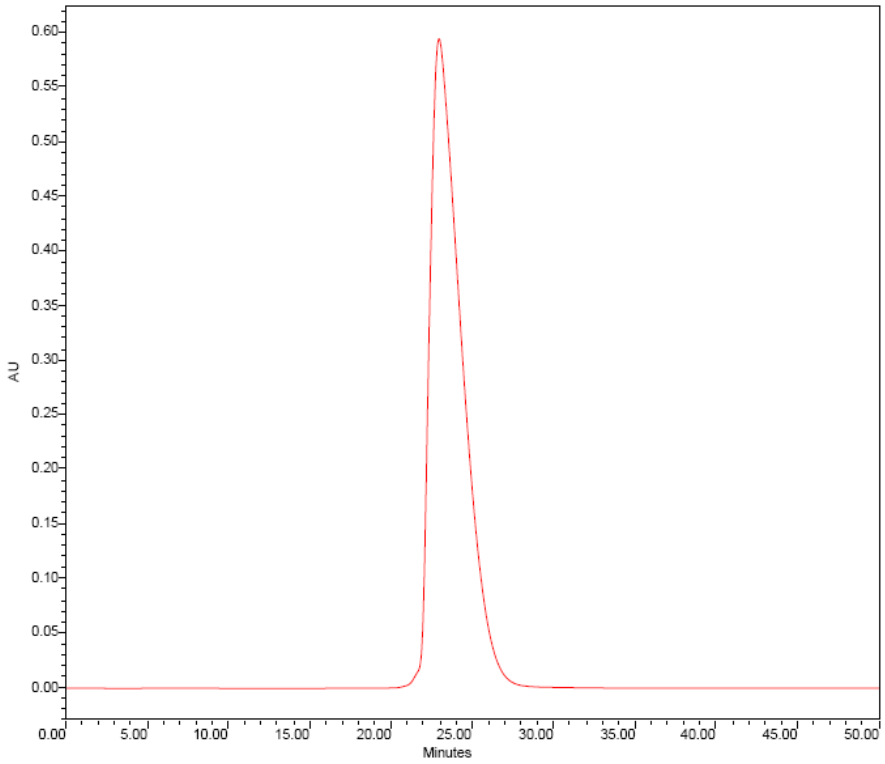


Synthesis and characterization of Fmoc-FY

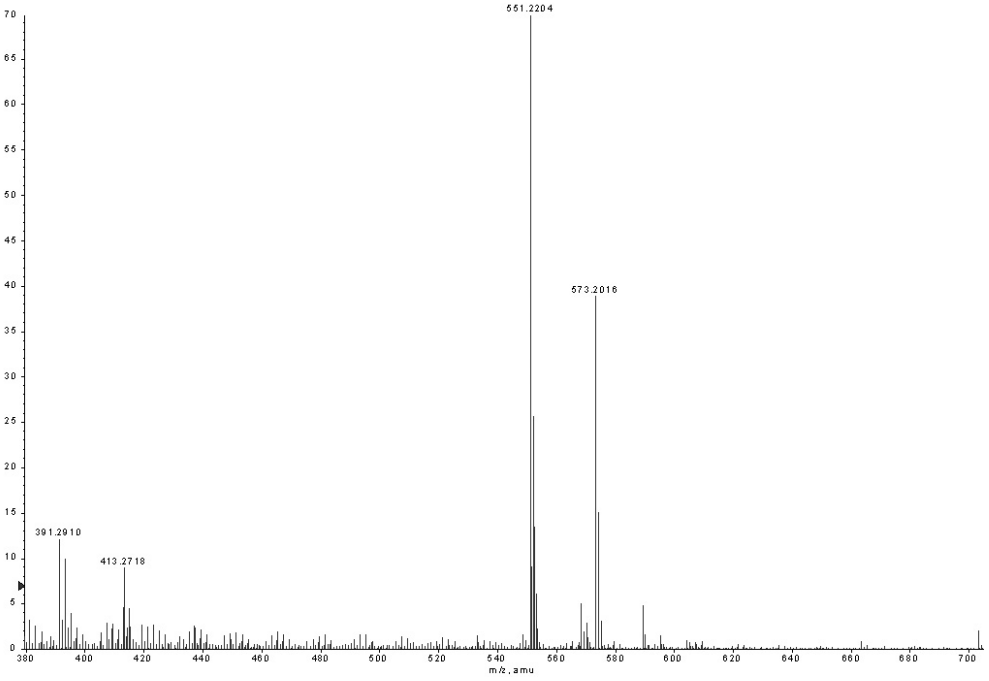
Fmoc-phenylalanine (387 mg, 1 mmol) was activated with NHS (115 mg, 1 mmol) and DCC (230 mg, 1.1 mmol) in 20 mL of $CHCl_3$ at room temperature for 2 h. The resulting solid was filtered and discarded, and the filtrate was dried by a rotary evaporator. Tyr(tBu)-OH (237.3 mg, 1 mmol) and $NaHCO_3$ (84 mg, 1 mmol) were dissolved in 30 mL of water/acetone (50/50). The solid of activated Fmoc-phenylalanine was dissolved in 150 mL of acetone and added to the above solution. Additional water was added to dissolve the reactants. The resulting clear solution was stirred at room temperature overnight. After the removal of the

solvent by blowing with compressed nitrogen, 200 mL of water was added. The precipitates were collected after centrifuging at 15000 rpm for 15 min and washed with diethyl ether. The precipitates separated from centrifuging were then dissolved in 15 mL of TFA in centrifuge tube and vortexed for 2 h. After the removal of TFA by compressed nitrogen, 35 mL of water was added. The precipitates were collected after centrifuging and then freeze-dried. The crude product was purified by RP-HPLC eluting with CH_3CN/water (40/60) containing 0.1% TFA. The purified sample was characterized by RP-HPLC on an analytical column with the detection wavelength of 265 nm (purity > 98%) and by MALDI-TOF-MS ($[M + H]^+$ calculated = 551.61, observed = 551.22; $[M + Na]^+$ calculated = 573.59, observed = 573.20).

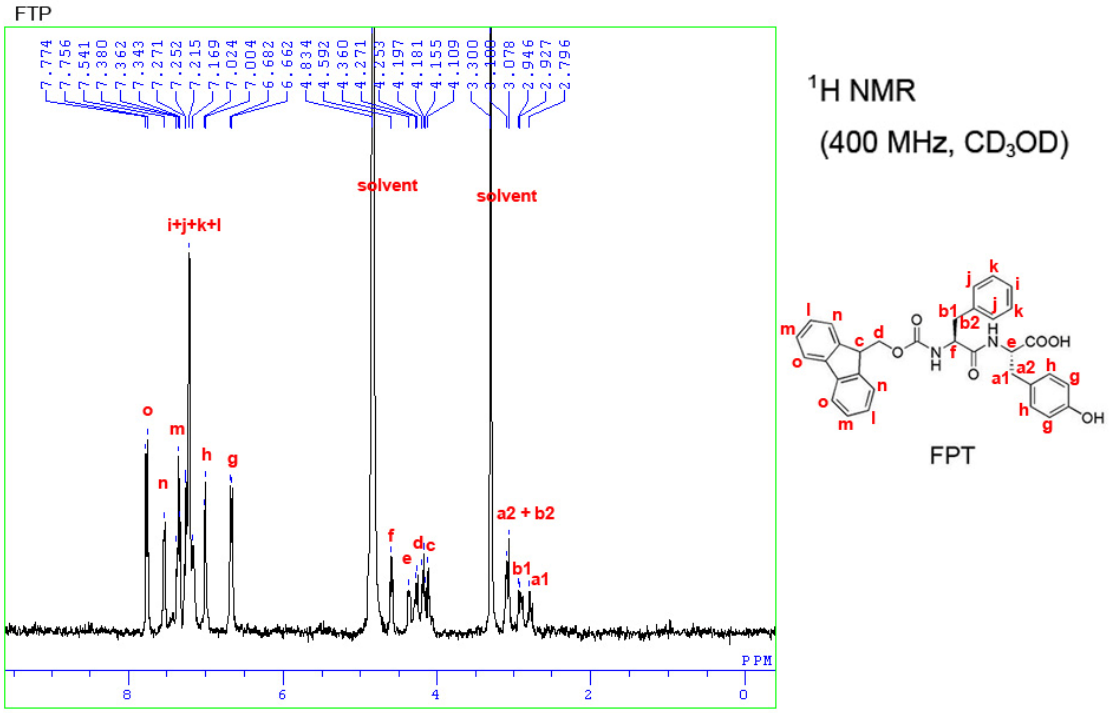
HPLC analysis of purified Fmoc-FY:



MS result of purified Fmoc-FY:



^{1}H NMR spectra of purified FTAEA:



Fabrication of FTAEA nanoparticles and two-component nanoparticles

FTAEA and Fmoc-FY were first dissolved in DMF to give stock solutions with concentrations of 40 mM and 80 mM, respectively. To fabricate FTAEA nanoparticles, FTAEA stock solution (0.25 μL) was added into 100 μL of water with vortexing. To produce two-component nanoparticles, Fmoc-FY stock solution (0.125 μL) was added into 100 μL of PB solution (20 mM, pH7.4) with vortexing and the solution was added into 100 μL of the solution of FTAEA nanoparticles drop by drop with vortexing. In this study, the final FTAEA concentration of FTAEA nanoparticle solution is 100 μM; the final FTAEA and Fmoc-FY concentration of two-component nanoparticle solution is 50 μM unless otherwise stated.

Analysis of the efficiency of FTAEA self-assembly

After preparing the FTAEA nanoparticle solution (50 μM), the solution (1.5 mL) was put into centrifugal filter units (Ultracel 3K, Millipore). The filtrate was collected after centrifuging at 4000 rpm for 20 min. Both the filtrate and the primary FTAEA nanoparticle solution were analyzed by using RP-HPLC on an analytical column with the detection wavelength of 265 nm. The experiments were performed in triplicate.

Dynamic light scattering

The number average diameter measurement was performed on Zeta Plus (Brookhaven Instruments Corp. (BIC)) with the use of the BIC Particle Sizing Software. The prepared solution (100 μL) was put into a cuvette (Eppendorf UVette) and tested at 25^{o}C with a detection angle of 90 degrees and a wavelength of 659 nm. Each sample was run for 10 times with 15 s per run. The data of each run were collected only when its baseline index was larger than or equal to 5. The experiments were performed in triplicate.

Cryo-TEM imaging

The measurements were conducted on an FEI/Philips Tecnai 12 BioTWIN transmission electron microscope, operating at 120 kV. Samples were prepared by a blotting procedure at room temperature and ~90% relative humidity. In a typical measurement, the sample solution (10 μL) was deposited on a copper grid coated by a lacey carbon film. Excess solution was blotted by a filter paper. The sample was immediately plunged into liquid propane cooled by liquid nitrogen, and the vitrified sample was transferred to the microscope with the protection of liquid nitrogen. The images were taken under low-dose conditions to minimize radiation damages to the samples.

Scanning electron microscopy

A 10μL aliquot of the solution of two-component nanoparticles was deposited and spread on a mica film. Excess solution was blotted by a filter paper. The sample was dried at room temperature and sputter-coated with gold. The imaging was conducted on a JEOL JSM 6700F SEM.

Fluorescence measurement

Fluorescence spectra were acquired using a Perkin-Elmer LS 50B spectrofluorometer with a Xenon discharge lamp excitation.

In Vitro Biocompatibility Study

The biocompatibility of the two-component nanoparticles stabilized by Fmoc-FY was evaluated by measuring the viability of NIH/3T3 cells in the presence of different concentrations of two-component nanoparticles. The viability of the cells was determined by MTT array. Cells were cultured in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% calf bovine serum (CBS) in a humidified atmosphere with 5% CO_2 at 37 ^{o}C. Cells were seeded on a 96-well plate at a density of 20,000 cells per well. After an overnight incubation, the growth medium was replaced with the mixture of 50 μL of serum-free medium and 50 μL of the solution of two-component nanoparticles at the indicated concentrations (the solution was supplemented with KCl and NaCl to give a concentration of 137 mM and 2.7 mM, respectively). After 24 hours of incubation, 10 μL of MTT solution (5 mg/mL in PBS, pH 7.4) was added to each well and the plate was then incubated at 37 ^{o}C for 4 hours. One hundred μL of SDS solution (10% w/v in 0.01 M HCl) was added to each well to dissolve the purple crystal of formazan. After an overnight incubation, the absorbance at 595 nm was measured with a plate reader (Wallac Victor^3 1420, PerkinElmer). The experiments were performed in triplicate. The percentage viability was calculated as follows:

$$\text{Viability (\%)} = (A_s - A_0) / (A_c - A_0) \times 100\%$$

where A_s is the absorbance value of the sample, A_c is the absorbance value of the control, and A_0 is the absorbance value of the background.

Entrapment of Nile red with two-component nanoparticles

A stock solution containing 20 mM FTAEA and 200 μM Nile red in DMF was firstly prepared. Then the stock solution (0.5 μL) was added into 100 μL of water with vortexing. Fmoc-FY stock solution (0.125 μL) was added into 100 μL of PB solution with vortexing and the solution was added into 100 μL of the above solution drop by drop with vortexing.

Figure S1-S6:

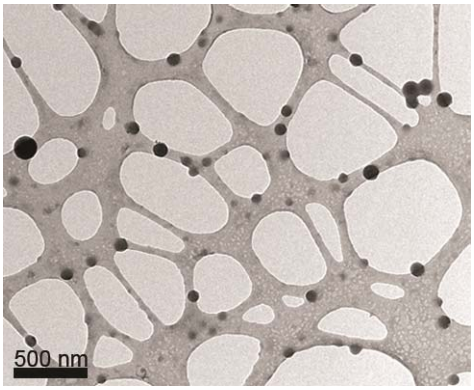


Figure S1 Cryo-TEM image of FTAEA nanoparticles.

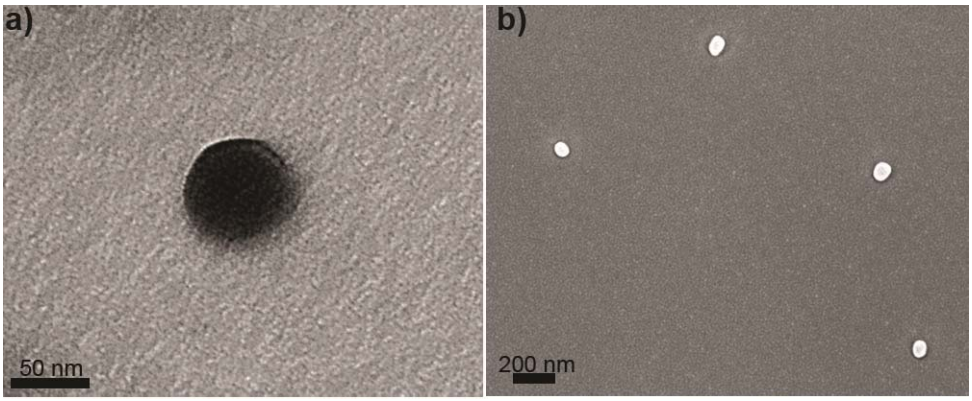


Figure S2 Cryo-TEM image (a) and SEM image (b) of two-component nanoparticles at a high magnification.

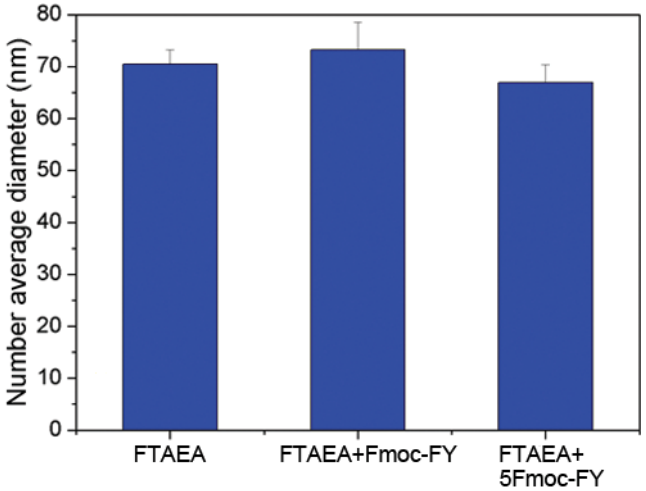


Figure S3 The number average diameters of FTAEA NPs and two-component NPs with the ratio of FTAEA to Fmoc-FY being 1:1 and 1:5. From two sample t-test, there is no significant difference between any two bars ($P > 0.05$). Mean ± SE ($n = 3$).

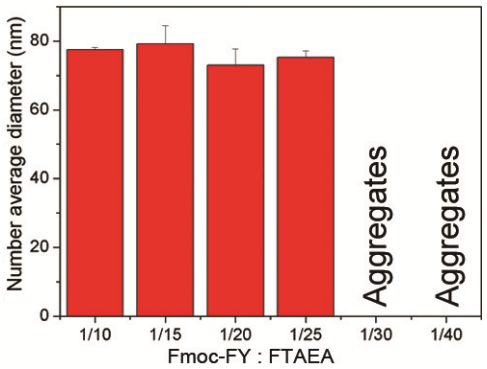


Figure S4 The number average diameters of two-component nanoparticles with the ratio of Fmoc-FY to FTAEA being from 1:10 to 1:25. At the ratio of 1:30 and 1:40, big aggregates were detected. The final FTAEA concentration of all samples is 50 μM. From two sample t-test, there is no significant difference between any two bars ($P > 0.05$). Mean ± SE ($n = 3$).

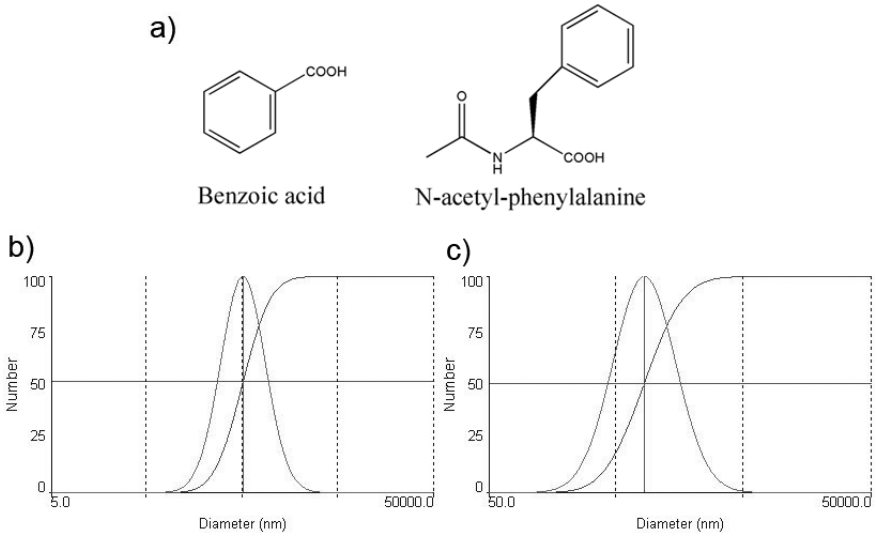


Figure S5 (a) Chemical structures of benzoic acid and N-acetyl-phenylalanine. Distribution of hydrodynamic diameters of FTAEA nanoparticles mixed with benzoic acid (b) and N-acetyl-phenylalanine (c). The results show that the number average diameters are 513.0 nm with the polydispersity of 0.350 (b) and 836.4 nm with the polydispersity of 0.391 (c). The final concentration of FTAEA, benzoic acid and N-acetyl-phenylalanine is 50 μM.

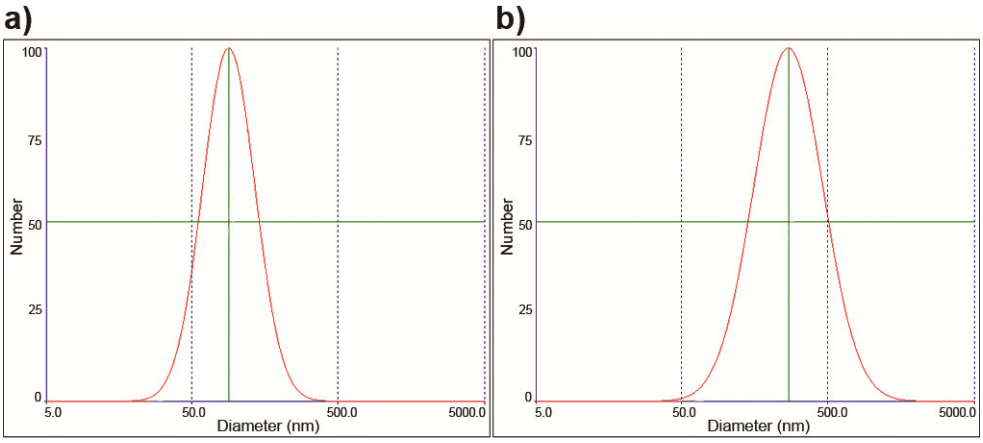


Figure S6 Distribution of hydrodynamic diameters of two-component nanoparticles incubated at room temperature for 6 weeks (a) and of FTAEA nanoparticles for 15 days (b). The results show that the number average diameters are 89.31 nm with the polydispersity of 0.206 (a) and 268.99 nm with the polydispersity of 0.366 (b).

ESI Video information:

File name: Fabrication of FTAEA nanoparticles

Short descriptive title: Nanoparticles self-assembled instantly with a facile method

Video legend: Spherical particles of around 70 nm are formed spontaneously by a simple trigonal Fmoc-conjugate (FTAEA). The nanoparticle preparation is facile, efficient and reproducible.