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Supramolecular Polymeric Peptide Amphiphile Vesicles For The Encapsulation of Basic Fibroblast Growth Factor

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S.1 Synthesis and characterization of 2

Synthesis of the hydroxyl group functionalised chain transfer agent, CTA-OH. The hydroxyl group-containing CTA-OH was synthesized in the following manner (Scheme S1). Ethanethiol (2.00 g, 32.19 mmol) was added to a stirred suspension of potassium phosphate (7.20 g, 33.92 mmol) in acetone (50 mL) and the reaction was stirred at room temperature for 45 min. Carbon disulphide (7.35 g, 96.53 mmol) was added and the resulting bright yellow solution was further stirred for 45 min. Then, 4-(chloromethyl)benzyl alcohol (5.04 g, 32.18 mmol) was added and the reaction mixture was left to stir at room temperature for 16 h. The reaction mixture was filtered and the volatiles were removed under reduced pressure. The solid residue was dissolved in ethyl acetate and the solution was subsequently washed with water and brine. The organic phase was dried over sodium sulphate and filtered. Then the solvent was evaporated and the crude product was purified by column chromatography using dichloromethane as eluent to give CTA-OH as yellow oil (6.24 g, 75%). ^{1}H NMR (400 MHz, $CDCl_3$): 7.30 (m, 4H), 4.67 (s, 2H), 4.61 (s, 2H), 3.38 (q, J = 7.4 Hz, 2H), 1.36 (t, J = 7.4 Hz, 2H).

Synthesis of hydroxyl group terminated poly(N-isopropyl acrylamide), PNIPAM-OH. N-isopropyl acrylamide (1.500 g, 13.256 mmol), CTA-OH (0.137 g, 0.530 mmol) and 4,4'-azobis(4-cyanopentanoic acid) (ACPA) (0.015 g, 0.054 mmol) were dissolved in 1,4-dioxane (6.6 mL) in a Schlenk tube and the solution was degassed by bubbling nitrogen for 40 min. The Schlenk tube was then sealed and heated in an oil bath set to 70 °C for 7 h. Upon completion, the polymerization mixture was cooled in liquid nitrogen and the polymer isolated by precipitation from cold diethyl ether. The resulting polymer was collected via decanting off the solution, dissolving the remaining polymer in tetrahydrofuran and then removing the solvent under reduced pressure. PNIPAM-OH was obtained as a waxy yellow solid. Yield: 1.457 g (89%). ^{1}H NMR (400 MHz, $CDCl_3$): 7.22-7.33 (br), 7.06-7.17 (m, 2H), 5.91-7.01 (br), 4.63 (s, 2H), 3.83-4.16 (br, 25H), 3.27-3.39 (m, 2H), 1.20-2.34 (br, 78H), 0.95-1.20 (br, 150H). M_n(NMR) = 3100 g/mol, M_n(GPC) = 2900 g/mol, M_w/M_n = 1.35.

Synthesis of methyl-viologen terminated poly(N-isopropyl acrylamide), PNIPAM-MV. PNIPAM-OH (0.183 g, 0.059 mmol) was added to a solution of MV-NCO (0.120 g, 0.178 mmol) in dry acetonitrile (5 mL). After addition of a drop of dibutyltin dilaurate, the resulting solution was stirred for 48 h at room temperature. The solvent was partially evaporated under reduced pressure and the crude was obtained by precipitation from cold diethylether. Then, the crude was washed with tetrahydrofuran and the washing solvent collected. Finally, the solvent was evaporated under reduced pressure to give PNIPAM-MV as a yellow powder. Yield: 0.168 g (75%). ^{1}H NMR (400 MHz, DMSO): 9.34 (d, J = 6.8 Hz, 2H), 9.29 (d, J = 6.8 Hz, 2H), 8.79 (d, J = 6.8 HZ, 2H) 8.75 (d, J = 6.8 Hz, 2H), 6.88-7.63 (m, 25H), 4.91-4.99 (m, 4H), 4.39-4.55 (m, 5H), 3.72-3.96 (m, 25H), 3.30 (br), 2.84-2.98 (m, 4H), 1.78-2.16 (br, 78H), 0.90-1.70 (br, 150H). M_n(NMR) = 3800 g/mol, M_n(GPC) = 3600 g/mol, M_w/M_n = 1.40.

S.2 Preparation and characterization of bFGF-loaded vesicles for the quantification of loading capacity

bFGF-loaded vesicle formulations were prepared by the temperature triggered process of forming vesicles. bFGF (100 μg/mL) containing heparin (1 mg/mL) was added dropwise to a stirring aqueous sus-

suspension of **1**+**2**⊂CB[8] complex vesicles (10 mg/mL) at 4 °C. The mixture was kept under magnetic stirring for 1 hour at 40 °C. Aggregates were removed by centrifugation, and unincorporated bFGF in the supernatant was removed by dialysis (MWCO 100 kDa). The purified bFGF-loaded vesicles were collected by freeze-drying. The bFGF loading efficiency was determined by ELISA. Particulate matter from the supramolecular complex was removed by centrifugation. Unloaded micelles were used as the blank sample. The loading efficiency of bFGF in the micelles was 34 %. All loading measurements were performed in triplicate.

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S.4 bFGF concentration measurements

The loss of bFGF by adsorption onto the walls of test tubes and cell culture plates was reduced by passivating the surfaces by an overnight soaking of BSA overnight in 1.0 mg/mL protein solution. The Quantikine human FGF basic immunoassay kit (96 wells) was used to measure the bFGF concentration in the samples. The Quantikine kit employs the enzyme-linked immunosorbent assay (ELISA), which is a widely accepted method for quantitative detection of the bioactive bFGF. In brief, standards and samples were pipetted into the microplate wells precoated with a monoclonal antibody specific for bFGF, and any bFGF present was bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked monoclonal antibody specific for bFGF was added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution was added to the wells, and color developed in proportion to the amount of bFGF bound in the initial step. Absorbance was read at 450 nm by a microplate reader with background subtraction at 570 nm. All data represented the mean of three measurements of three different trials, and results were reported as the means and standard deviations of these measurements.

S.5 Cell culture studies

NIH 3T3 cell line was used to study the effect of the vesicle encapsulation on bFGF delivery. The cells were incubated in tissue culture flasks at 37 °C in a humidified atmosphere of 5% CO2 and 95% air incubator before seeding in cell culture plates for further in vitro experiments. The cell culture medium consisted of Dulbecco's Modified Eagle Medium (DMEM) with 10% fetal bovine serum (FBS), penicillin (50 U/mL), and streptomycin (0.05 mg/mL). The cell density was 5000 cells per well in a 96-well plate; the volume of the medium was 200 μL; and experiment duration without medium refreshment was 3 days. For the purpose of attachment, the cells were seeded in wells filled with 5% FBS DMEM. One day

after (day 0), the medium was replaced with serum-free DMEM, and tested dispersions of microcapsules or bFGF solutions were added. The effect of bFGF on cell proliferation was evaluated three days later (day 3) using the calorimetric MTS assay designed to measure the activity of a dehydrogenase enzyme in metabolically active cells. The absorbance corresponding to the amount of active cells was measured at 492 nm by a microplate reader.

S.6 Turbidity measurements for LCST

UV/vis spectra were recorded on a Varian Cary 100 Bio UV-Vis spectrophotometer. The turbidity was measured at a wavelength of 600 nm.

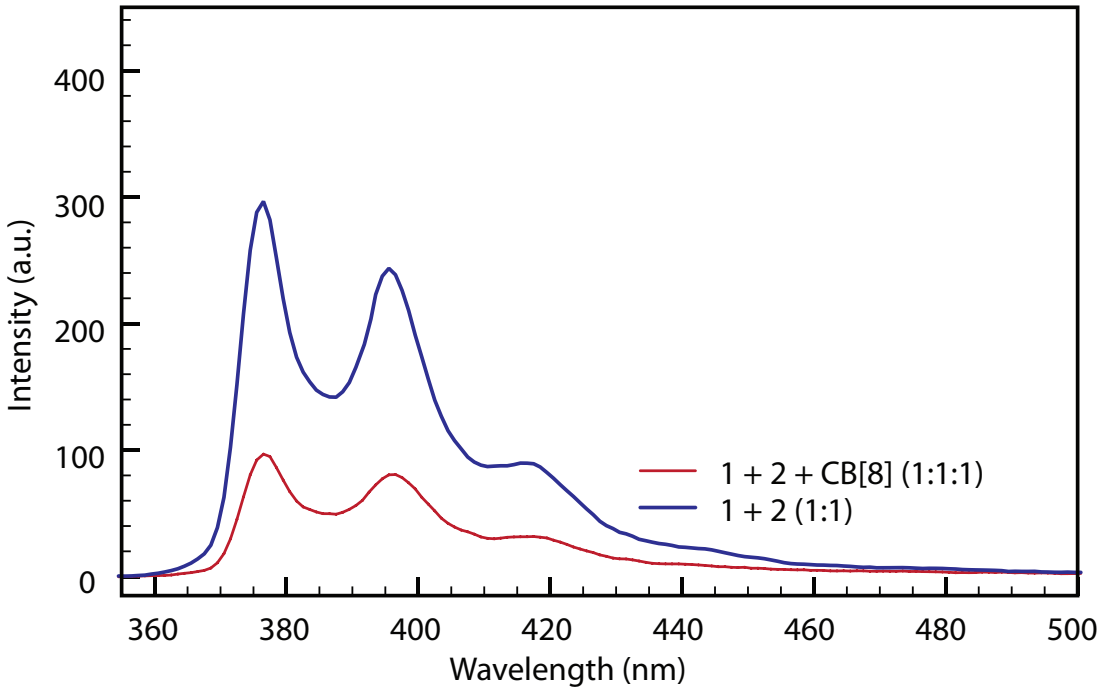

Figure S1: Fluorescence profile of ternary complexes (0.05 mM) compared with polymeric mixtures at 37 °C, pH 7.4. (Excitation wavelength = 303nm)

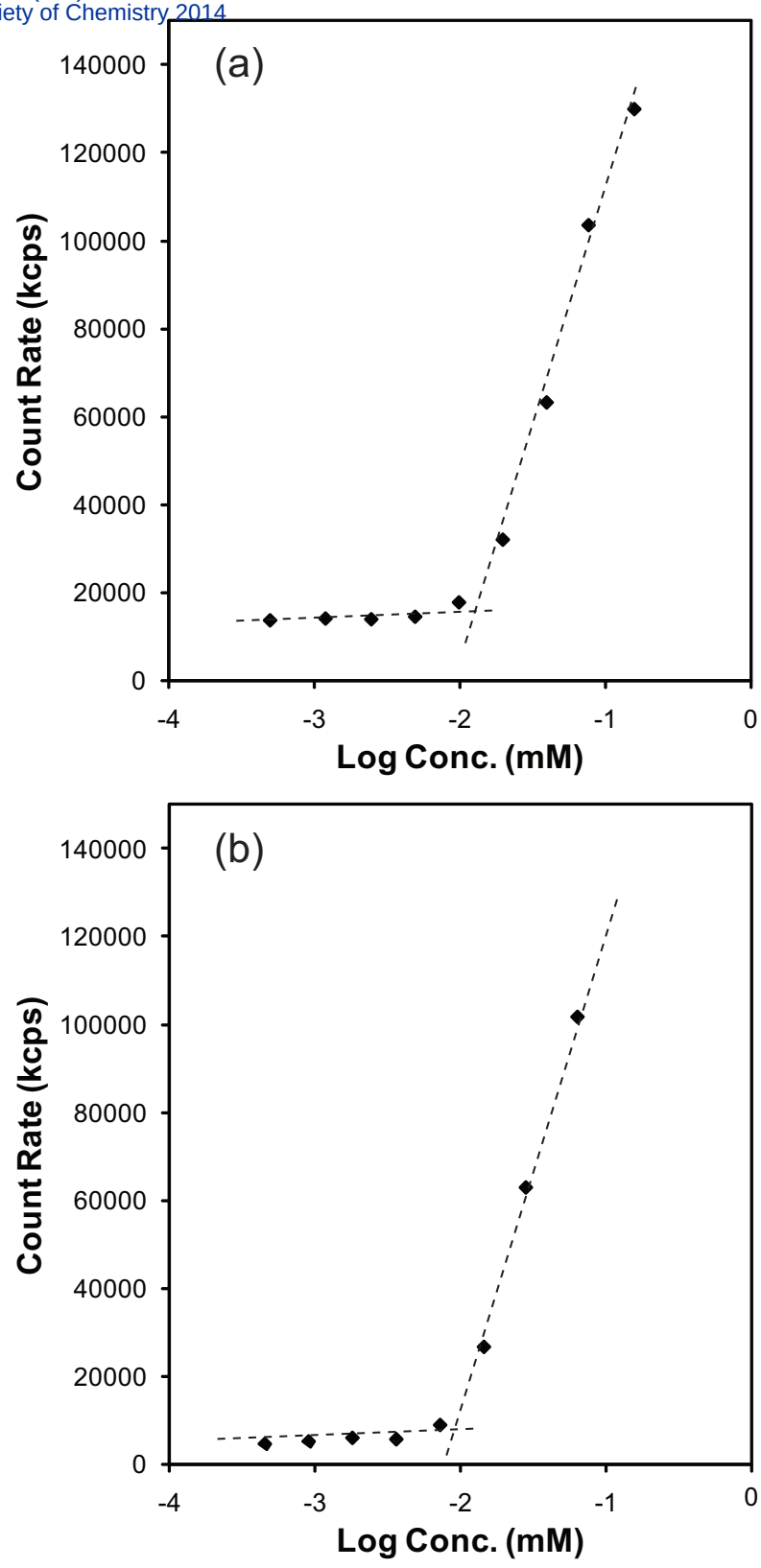

Figure S2: Determination of the CAC of the supramolecular vesicles in (a) deionised water and (b) 1X phosphate buffer saline (PBS) solution containing 1% fetal bovine serum (FBS) at pH 7.4

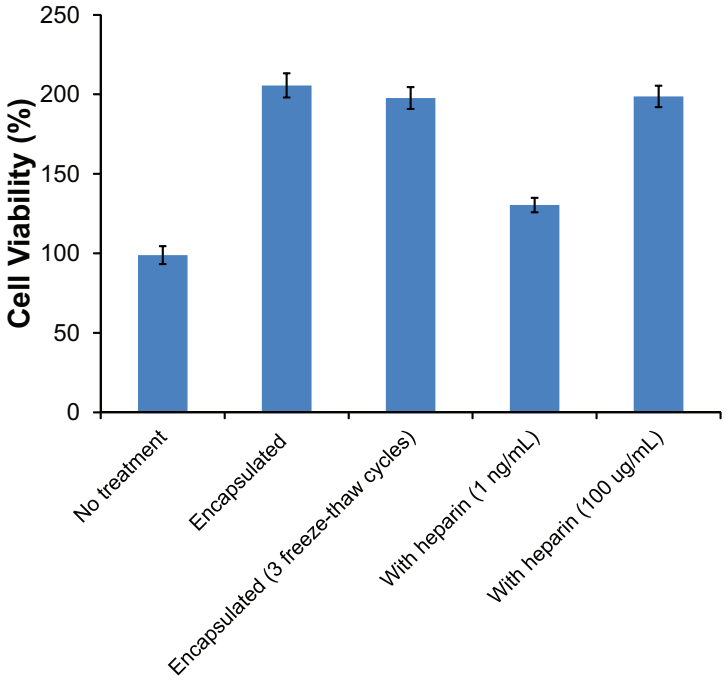

Figure S3: Effect of freeze-thaw denaturing conditions as compared to heparin treatment on the bioactivity of bFGF encapsulated in the vesicles.

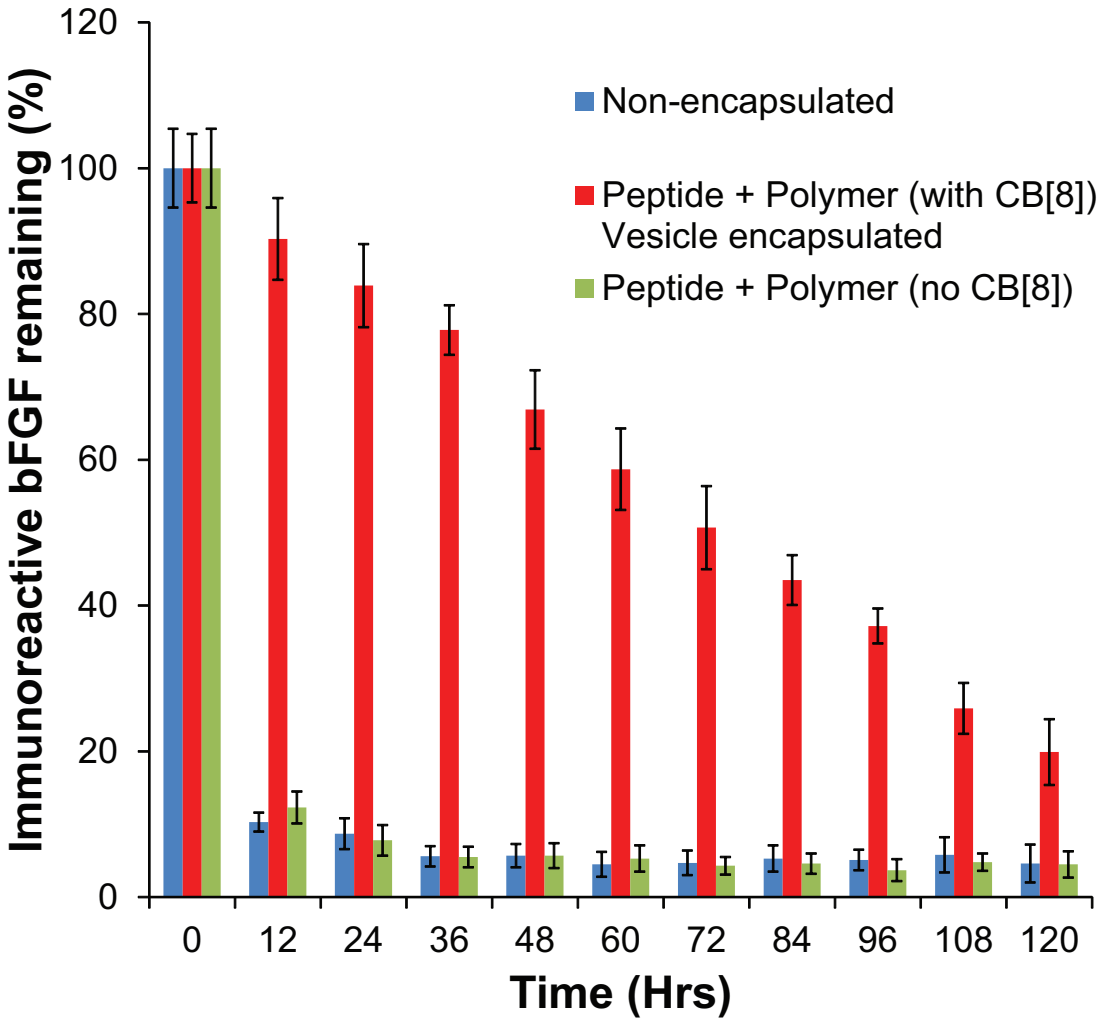

Figure S4: Assessment of immunoreactive bFGF in various solutions with and without CB[8].

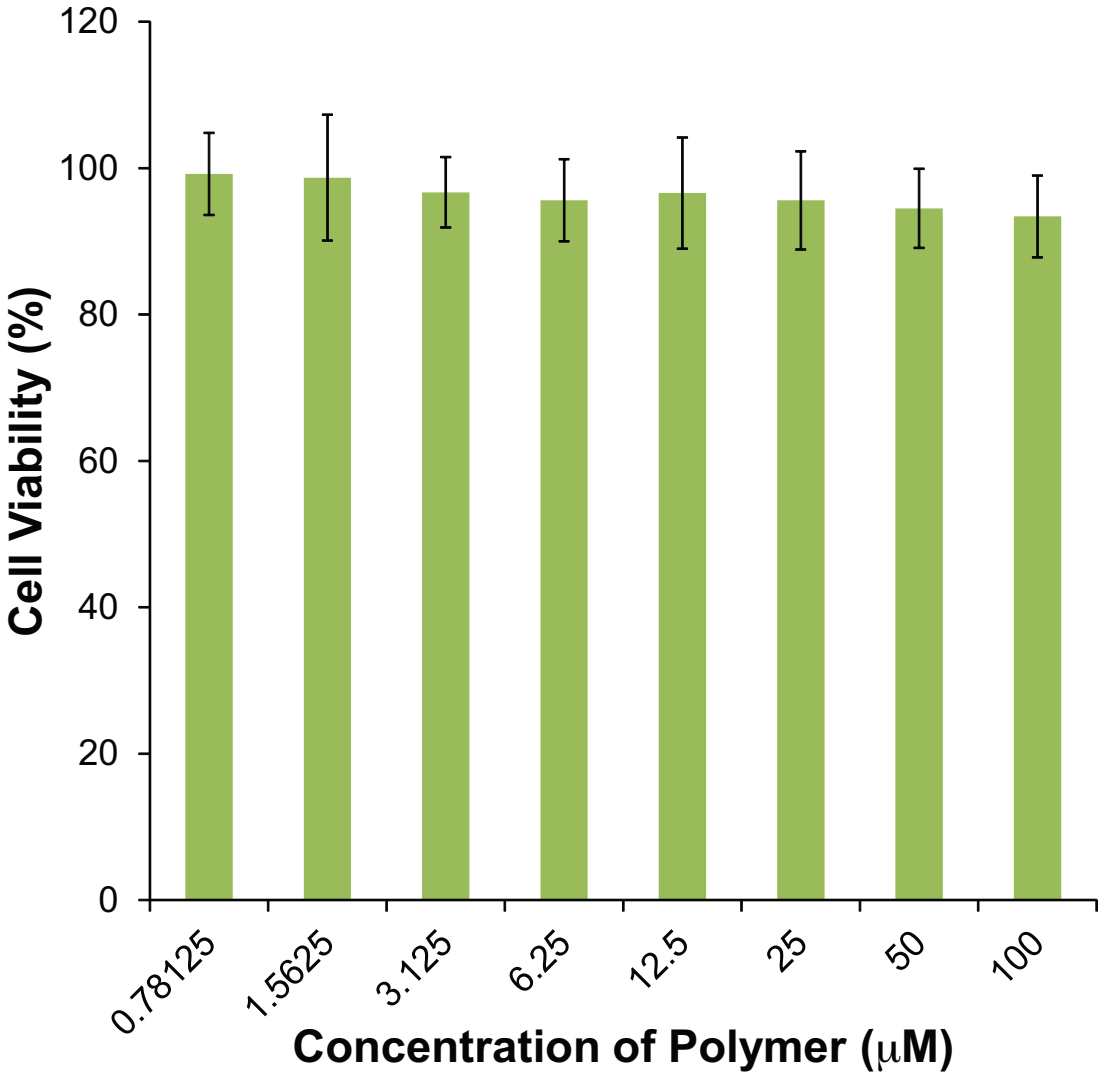

Figure S5: Cytotoxicity of the ternary complexes at various concentrations using NIH 3T3 cells.

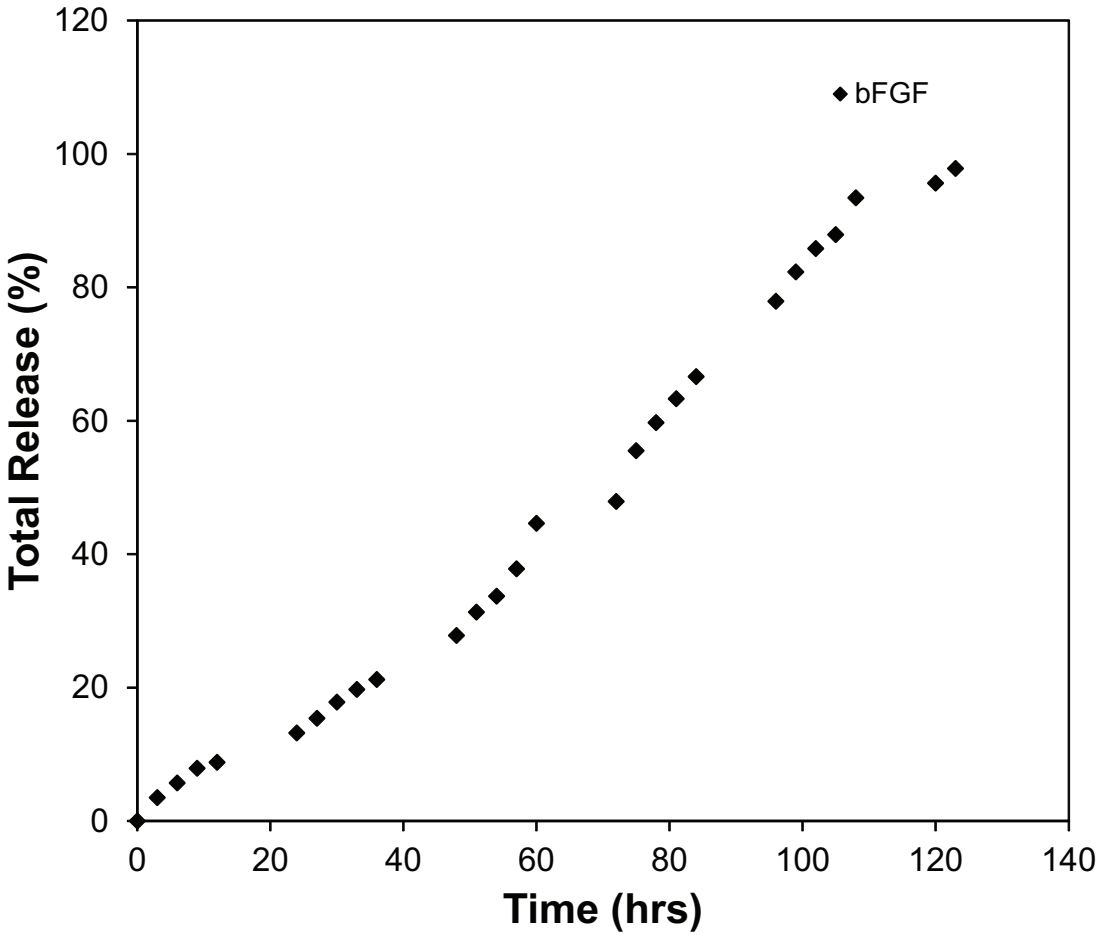

Figure S6: bFGF release (37 ^{o}C) from the vesicles made from the ternary complexes.

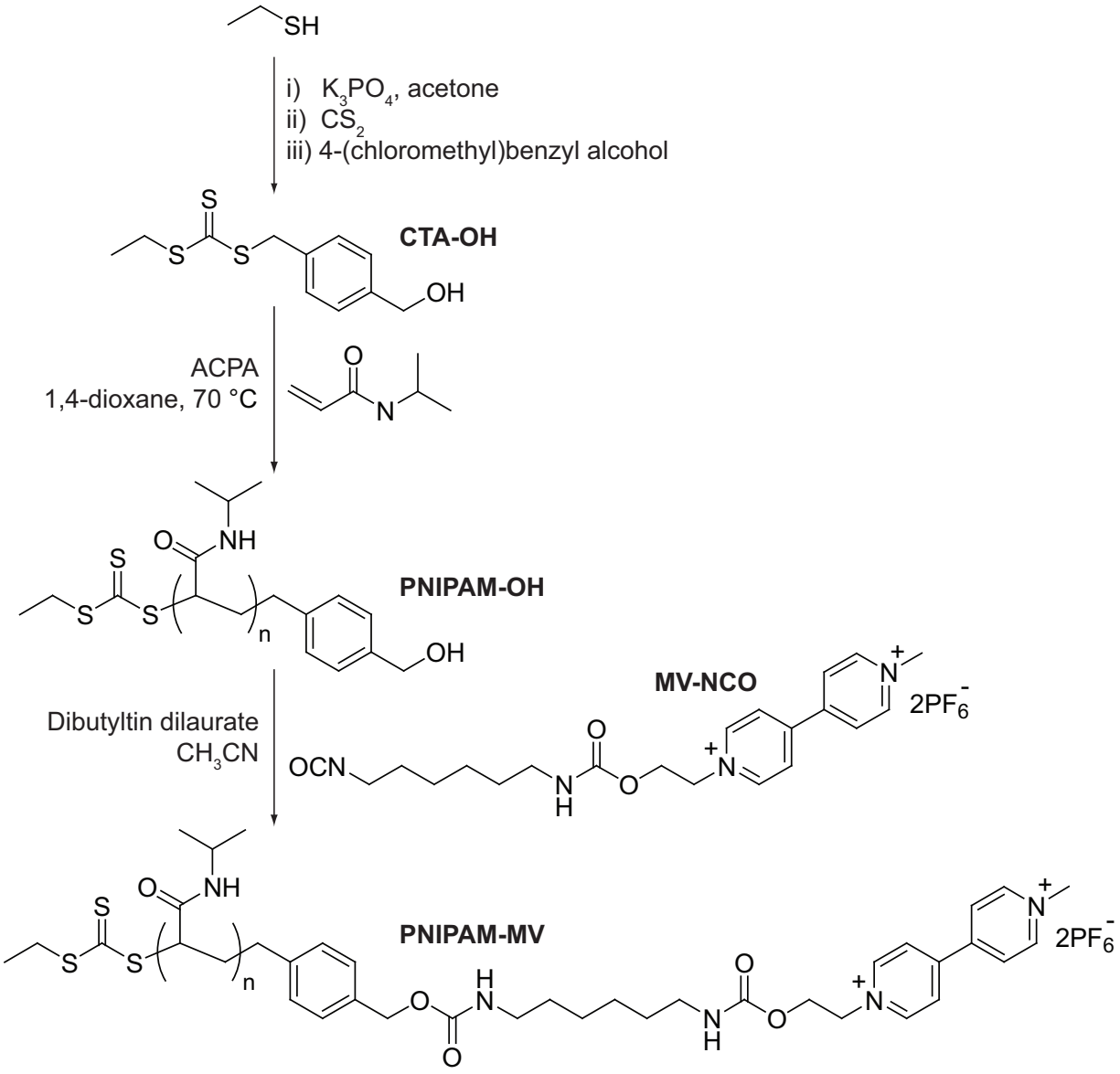

Figure S7: Reaction scheme for the synthesis of PNIPAAm-MV.