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# **ARTICLE TYPE**

# Highly Emissive PEG-Encapsulated Conjugated Polymer Nanoparticles

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## **5 Supporting information**

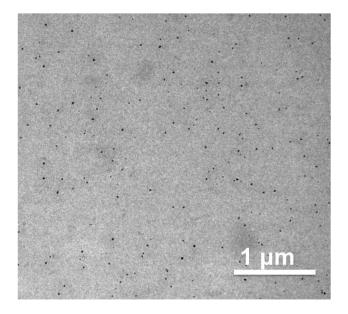


Figure S1. TEM image of PEG-PFBD CPNs showing no aggregation of the nanoparticles in solution prior to deposition.

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### Fluorescence Stability of PEG-PFBD CPNs as a Function of pH

 $1 \times PBS$  solutions with different pH values were prepared from pH = 2 to pH = 12 in 1 mL volume each. A stock solution of PEG-PFBD CPNs with absorbance equal to 0.1 was diluted 10 times into each of the pH buffer solutions. Fluorescence spectra of the resulting CPN solutions in various pH conditions were measured using a LS-55 spectrofluorometer (Perkin Elmer, USA) <sup>15</sup> using an excitation wavelength of 460 nm (Fig. S1a). OriginPro8 ® software was used to compute the total fluorescence intensity under each spectrum, and the data were normalized using the highest value at pH = 8 to obtain Fig. S2b.

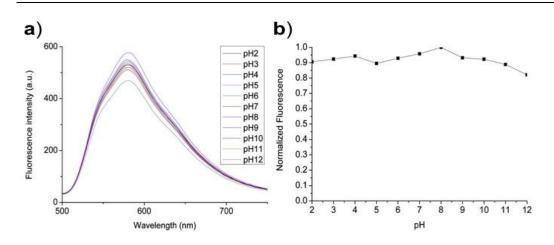
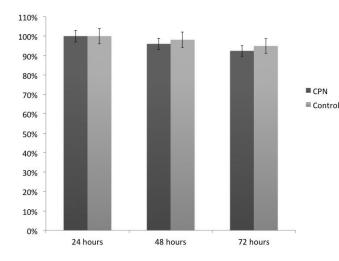


Figure S2. a) Fluorescence spectra of the PEG-PFBD CPNs at different pH values from pH = 2 to pH = 12. b) Normalized fluorescence obtained from a) as a function of pH.

#### 5 Effect of PEG-PFBD CPNs on the Viability of NIH/3T3 Cells over the Course of 72 Hours

NIH/3T3 fibroblast cells were provided by American Type Culture Collection and cultured in folate-free RPMI 1640 medium containing 10% fetal bovine serum and 1% penicillin–streptomycin at 37 °C in a humidified environment containing 5% CO<sub>2</sub>. Before experiment, the cells were precultured until confluence was reached. MTT and trypsin solutions were purchased from Sigma-Aldrich. MTT assays were performed to assess the metabolic viability of NIH/3T3 fibroblast cells. The NIH/3T3 cells

- <sup>10</sup> were seeded in 96-well plates (Costar, IL, USA) at an intensity of  $4 \times 10^4$  cells per mL. After 24 h incubation, the medium was replaced by PEG-PFBD CPN suspension at a concentration of 10 nM, and the cells were then incubated for 48 h and 72 h, respectively. After the designated time intervals, the wells were washed twice with 1× PBS buffer, and 100mL of freshly prepared MTT (0.5mg mL<sup>-1</sup>) solution in culture medium was added to each well. The MTT medium solution was carefully removed after 3 h incubation in the incubator. Isopropanol (100mL) was then added into each well, and the plate was gently
- <sup>15</sup> shaken for 10 min at room temperature to dissolve all precipitates formed. The absorbance of MTT at 570nm was monitored by the microplate reader (Genios Tecan). Cell viability was expressed by the ratio of absorbance of the cells incubated with CPN suspension to that of the cells incubated with culture medium only. Figure S3 shows the cell viability after incubation with PEG-PFBD suspension at a concentration of 10 nM (cell imaging concentration) for 24, 48 and 72 hours, respectively. The metabolic viability of NIH/3T3 cells does not change significantly after incubation with CPN-loaded suspension in comparison to control cells (without the addition of CPNIs), which indicated the law superspine of CPNIs to NIH/3T3 for cells within the tested
- <sup>20</sup> cells (without the addition of CPNs), which indicated the low cytotoxicity of CPNs to NIH/3T3 fibroblast cells within the tested period.



<sup>25</sup> Figure S3. Metabolic viability of NIH/3T3 fibroblast cells after incubation with the PEG-PFBD CPN suspension (black) and without CPN suspension (grey) for 24, 48, and 72 h, respectively.

#### Notes

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