Supporting Information

Phalloidin-Functionalized Hyperbranched Conjugated Polyelectrolyte for Filamentous Actin Imaging in Living Hela Cells

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Calculation of molecular weight and quaternization degree for HCPE-COOH. From the ^{1}H NMR spectrum of **P0**, the ratio of the integrated area for the peak at 3.67 ppm (the proton resonance of residual alkynes) to that at 2.00 ppm (the proton resonance of methylene groups next to the 9-position of fluorene) is ~0.14, which indicates that the percentage of triple bond involved in "new" benzene formation is 72%. According the equations reported by Tang et. al,[1] the number of newly formed benzene ring is estimated to be 12, and the degree of polymerization is 25, which yields the number average molecular weight of **P0** as ~16000. Therefore, there are ~14 residual alkynes for further functionalization. These calculations are based on the assumption that there is no or low amount of loops formed during polymerization, which is reasonable considering the steric effect of the monomer and mathematical probabilities.

On the other hand, from the ^{1}H NMR of HCPE-COOH, the ratio of the integrated area for the peak at 2.28 ppm (the proton resonance of methylene groups next to the 9-position of fluorene) to that for the peak at 3.00 ppm (-N(C$\underline{H}_3$)$_3$Br) is ~0.24, which indicates that the quaternization degree is ~93%. In addition, the ratio of the integrated area for the peak at 8.55 ppm (the proton next to the nitrogen atom of the triazole group) to that at 2.28 ppm (the proton resonance of methylene groups next to the 9-position of fluorene) is ~0.14, which suggests that almost all the residual alkynes are converted to triazole groups to yield ~14 PEG-COOH/**P1**. The number average molecular weight of HCPE-COOH is thus estimated to be ~26000.

Calculation of phalloidin immobilized on HCPE nanospheres. As described in the experimental section, after phalloidin modification and dialysis, the collected MilliQ water solution was concentrated to 10 mL through freeze-drying. A standard curve was constructed using a series of known concentrations of phalloidin analyzed through HPLC (Agilent LC 1100) using C18 column at 220 nm UV absorption. The mobile phase was composed of acetonitrile and water (50/50, v/v) and delivered at a flow rate of 1 mL/min. The dialysis solution was then analyzed using HPLC under the same experimental conditions and the concentration of free amino-phalloidin was derived from the standard curve. The total amount of amino-phalloidin reacted with HCPE-COOH was then calculated as follows.

The amount of amino-phalloidin (molecular weight is 959.1) used for reaction was 0.76 mg, and the amount of free amino-phalloidin in dialysis solution was determined to be 0.031 mg. As a result, 0.729 mg amino-phalloidin was immobilized on 3.96 mg HCPE nanospheres (number average molecular weight is ~26000), the amount of phalloidin molecule conjugated on each HCPE nanosphere was calculated to be ~5.

$$Phalloidin/HCPE = \frac{\text{Total Number of Phalloidin Reacted with HCPE}}{\text{Total Number of HCPE}}$$

$$= \frac{\frac{0.729}{959.1}}{\frac{3.96}{26000}} = 5$$

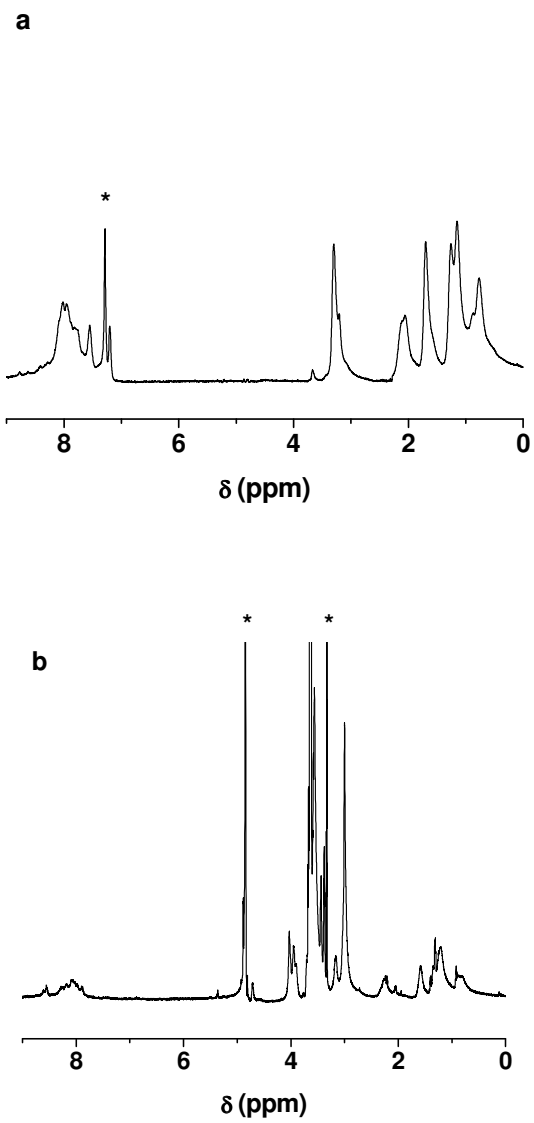

Figure S1. ^{1}H NMR spectra of (a) **P0** and (b) HCPE-COOH. The solvent peak at 7.29 ppm corresponds to the chemical shift of chloroform-d (a), and peaks at 4.85 ppm and 3.33 ppm correspond to chemical shifts of H_2O and methanol-d (b).

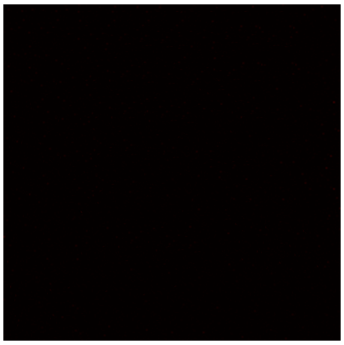

Figure S2. Confocal image of Hela cells without HCPE-phalloidin incubation (λ_{ex} = 405 nm, 1 mW laser power).

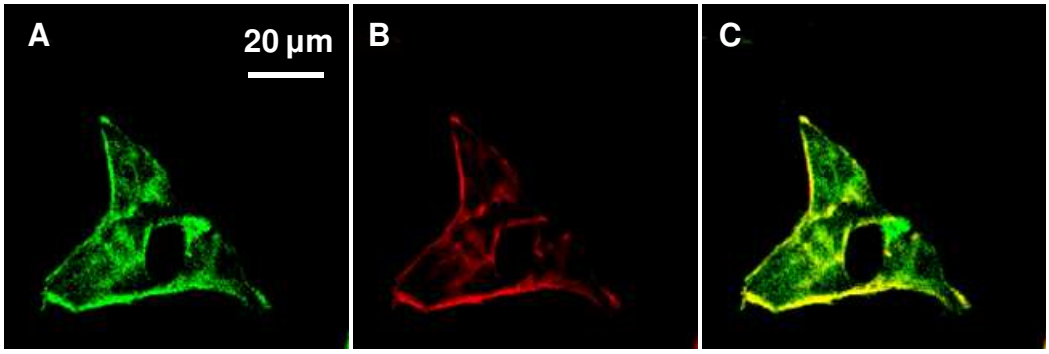

Figure S3. Colocalization of HCPE-phalloidin (A, λ_{ex} = 405 nm, 1.25 mW laser power, 490-560 nm band pass filter) and Alexa Fluor ® 594-phalloidin (B, λ_{ex} = 543 nm, 1 mW laser power, 565-655 nm band pass filter). Image C is the overlapped image of A and B. Hela cells are first incubated with 1 μg/mL of HCPE-phalloidin for 2 h at 37 °C. After fixation, the cells are further stained with 0.1 μM of Alexa Fluor ® 594-phalloidin in 1× PBS buffer for 40 min at room temperature. All images share the same scale bar.

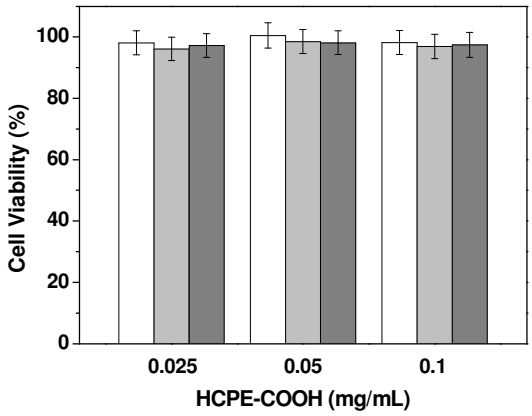

Figure S4. Metabolic viability of Hela cells treated with the HCPE-COOH solutions at the concentrations of 0.025, 0.05, and 0.1 mg/mL for 12 h (blank), 24 h (gray), and 48 h (dark gray), respectively.

Reference

(1) Liu, J. Z.; Zheng, R. H.; Tang, Y. H.; Häussler, M.; Lam, J. W. Y.; Qin, A. J.; Ye, M. X.; Hong, Y. N.; Gao, P.; Tang, B. Z. *Macromolecules* **2007**, *40*, 7473.