Electronic Supplementary Information (ESI)
for

A Synergistic Polyphosphoester-Based Co-delivery System of an Anticancer Drug Doxorubicin and the Tumor Suppressor Gene P53 for Lung Cancer Therapy

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1. Synthesis of Diblock Copolymer Precursor mPEG-*b*-PBYP

Polyphosphoesters (PPEs) were synthesized by ring opening (ROP) reaction according to previous publcation.[S1, S2] mPEG (0.82 g, 0.16 mmol) were added to a 50 mL dry branch flask and toluene was used as azeotropic water stripping agent. After two times of azeotropic distillation, BYP (2.12 g, 11.9 mmol) and DBU (0.049 g, 0.32 mmol) were added and dissolved in 7 mL of anhydrous CH_2Cl_2. Followed by three exhausting-refilling nitrogen cycles, the reaction was conducted at 25 °C for 30 min. The solvent was concentrated and precipitated with 100 mL cold diethyl ether/methanol (10/1) twice. The white sticky solid was collected and dried under vacuum at 25 °C to a constant weight (mPEG-*b*-PBYP, 2.64 g, yield: 89.8%).

2. Synthesis of DOX Derivative DOX-*hyd*-N_3.

DOX-*hyd*-N_3 was prepared and purified according to the literature.[S3] In a 50 mL dry round bottomed flask, 6-bromohexanoic acid (2.35 g, 11.24 mmol) and sodium azide (NaN_3, 1.83 g, 28.15 mmol) wee dissolved with 15 mL DMF and reacted for 12 h at 60 °C. The crude product was filtered with a short column of neutral aluminum oxide to remove the unreacted NaN_3 and then remove the solvent (DMF) by decompression. Subsequently, the crude product was dissolved with 20 mL dichloromethane (CH_2Cl_2) and washed three times with 40 mL Milli-Q water. The organic layer was collected and dried over anhydrous Na_2SO_4 for 2 h and evaporated to provide a product. After drying under vacuum at 25 °C for 24 h, 6-azidohexanoic acid methyl ester was obtained (1.14 g, yield: 66.8%).

6-azidohexanoic acid methyl ester (0.54 g, 3.17 mmol), hydrazine hydrate 85% (4.70 g, 79.4 mmol) and 20 mL THF were added to a round bottomed flask, stirred evenly and refluxed for 12 h at 80 °C. After removing the solvent by rotary evaporation, the crude product was dissolved in CH_2Cl_2 and washed with sodium chloride (NaCl) aqueous solution for twice. The organic phase was dried over anhydrous Na_2SO_4. The filtrate was concentrated and dried under vacuum at 25 °C for 24 h to get the 6-azidehexanohydrazine (0.29 g, yield: 53.5%).

Finally, 6-azidehexanohydrazine (102 mg, 0.60 mmol), DOX·HCl (115.9 mg, 0.20

mmol), anhydrous Na_2SO_4 (103 mg, 0.73 mmol) and 30 mL anhydrous methanol (CH_3OH) were added into the round bottom flask with condensation reflux device. Then added 2~3 drops of glacial acetic acid, fully stirred evenly and reacted at 60 °C for 24 h in dark. At the end of the reaction, the product was precipitated three times with diethyl ether, and the precipitate was obtained by centrifugation. After 24 h of vacuum drying, the red powder product was obtained (DOX-*hyd*-N_3, 93.5 mg, yield: 62.4%).

3. Measurement of critical aggregation concentration (CAC)

The critical aggregation concentration (CAC) was measured by a fluorescence probe method on a fluorescence spectrophotometer (Cary Eclipse, Agilent Technologies) and pyrene was used as the probe. Typically, a predetermined pyrene solution in acetone was added into a series of ampoule bottles and then acetone was removed under reduced pressure. The bottles were separately added 5 mL of the copolymer solution with different concentrations. Then the mixture was sonicated for 20 min and stirred for 48 h at room temperature before measurement. The solutions were analyzed on a fluorescence spectrophotometer. The excitation wavelength was 335 nm and the emission spectra were recorded ranging from 350 nm to 500 nm with a 2.5 nm slit width at medium voltage. From the emission spectra, the intensity ratio (I_3/I_1) of the third bond (383 nm, I_3) to the first bond (372 nm, I_1) was analyzed as the function of the logarithm concentration of the copolymer solution. The CAC value was dependent on the intersection of the two lines in the plot of intensity ratio (I_3/I_1) versus the copolymer concentration.

4. Supplementary Figures:

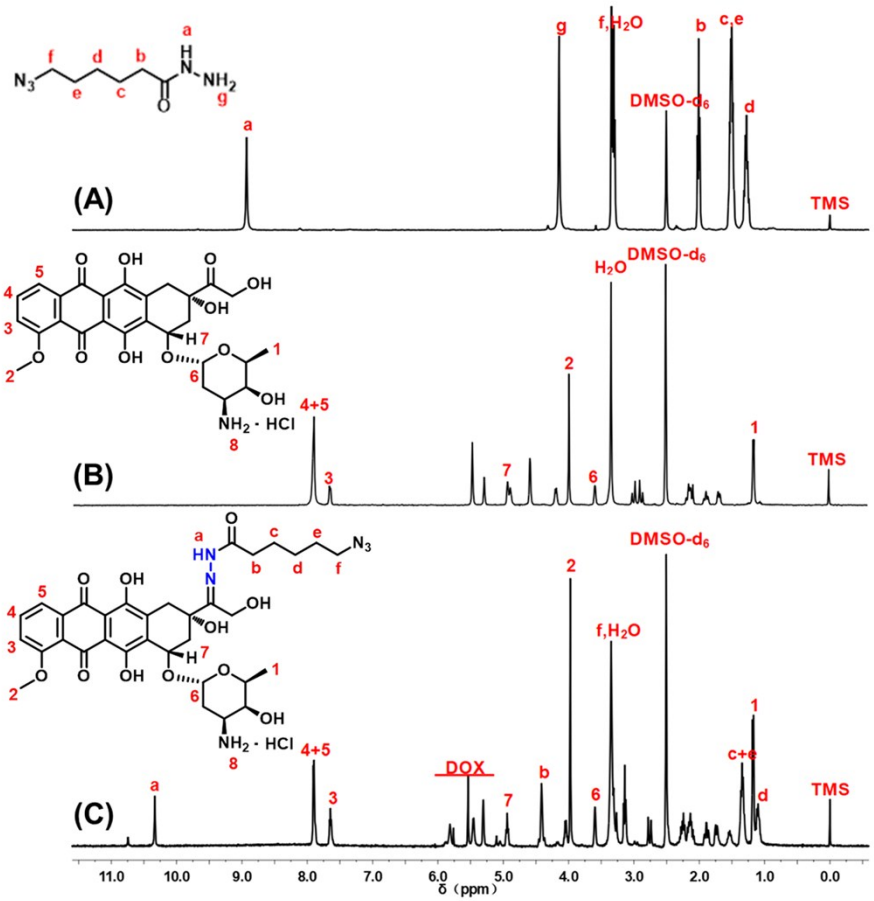

Figure S1. ^{1}H NMR spectra of (A) 6-azidehexanohydrazine, (B) DOX and (C) DOX-*hyd*-N_3 in DMSO-d_6.

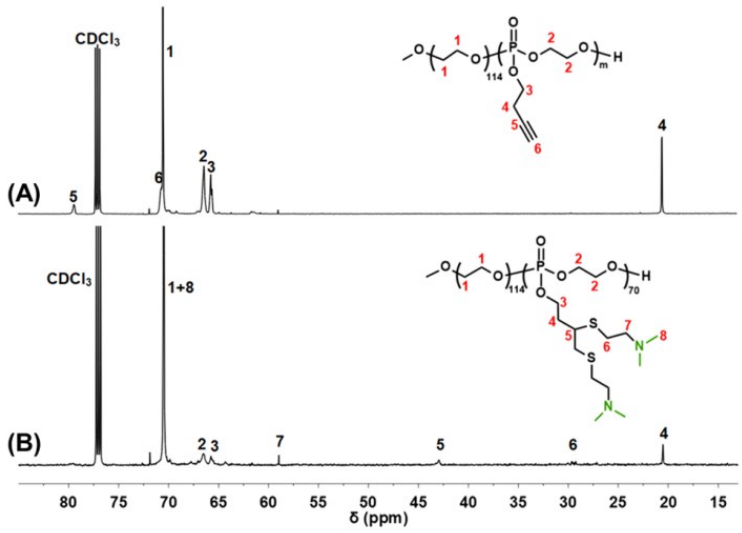

Figure S2. ^{13}C NMR spectra of (A) mPEG-*b*-PBYP and (B) mPEG-*b*-PBYP-*g*-DAE in $CDCl_3$.

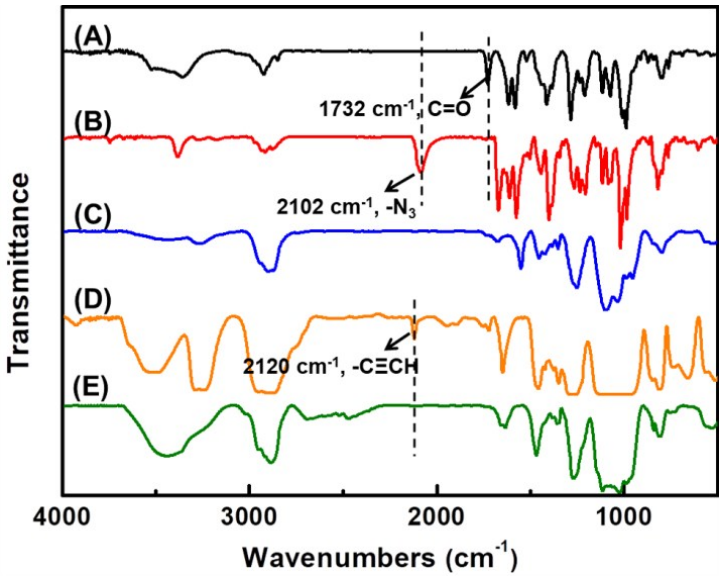

Figure S3. FT-IR spectra of (A) DOX, (B) DOX-*hyd*-N_3, (C) mPEG-*b*-PBYP-*hyd*-DOX, (D) mPEG-*b*-PBYP and (E) mPEG-*b*-PBYP-*g*-DAE.

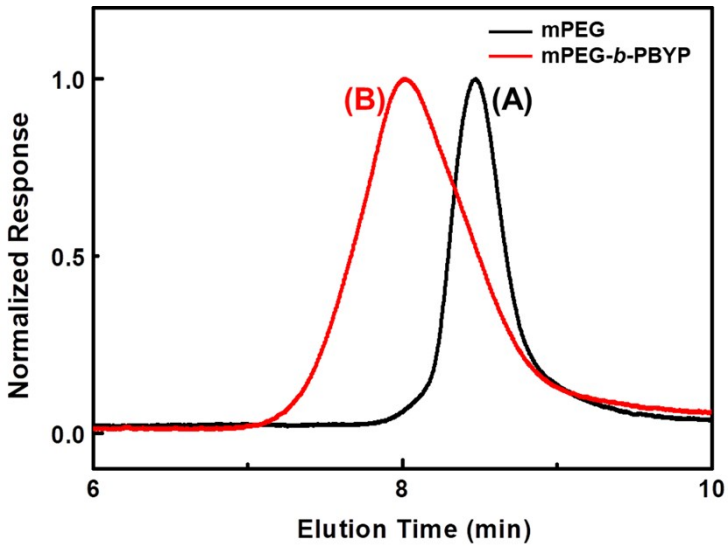

Figure S4. GPC curves of (A) mPEG ($\overline{M}_n$=7500 g mol^{-1}, PDI=1.02) and (B) mPEG-*b*-PBYP ($\overline{M}_n$=15100 g mol^{-1}, PDI=1.30) in DMF.

Table S1. Molecular weight and molecular weight distributions of mPEG-*b*-PBYP in different sample entry.

Entry	Mole ratio mPEG : BYP	$\overline{M}_{n,NMR}$ [a] (g mol^{-1})	$\overline{M}_{n,GPC}$ [b] (g mol^{-1})	PDI [b]
mPEG-*b*-PBYP$_{34}$	1:60	10900	10200	1.12
mPEG-*b*-PBYP$_{58}$	1:70	15000	11800	1.11
mPEG-*b*-PBYP$_{26}$	1:70	9500	10500	1.23
mPEG-*b*-PBYP$_{63}$	1:70	15800	10400	1.34
mPEG-*b*-PBYP$_{86}$	1:70	19900	15100	1.30

[a] Calculated based on ^{1}H NMR analysis in $CDCl_3$.

[b] Determined by GPC with DMF as the eluent and polystyrene as the standards.

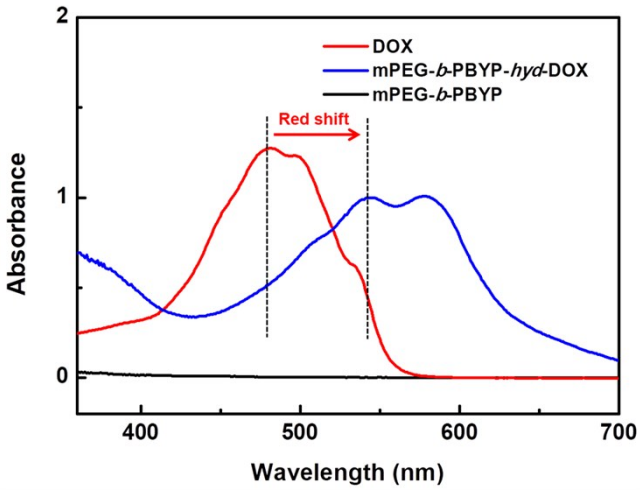

Figure S5. UV-Vis spectra of free DOX, mPEG-*b*-PBYP and mPEG-*b*-PBYP-*hyd*-DOX.

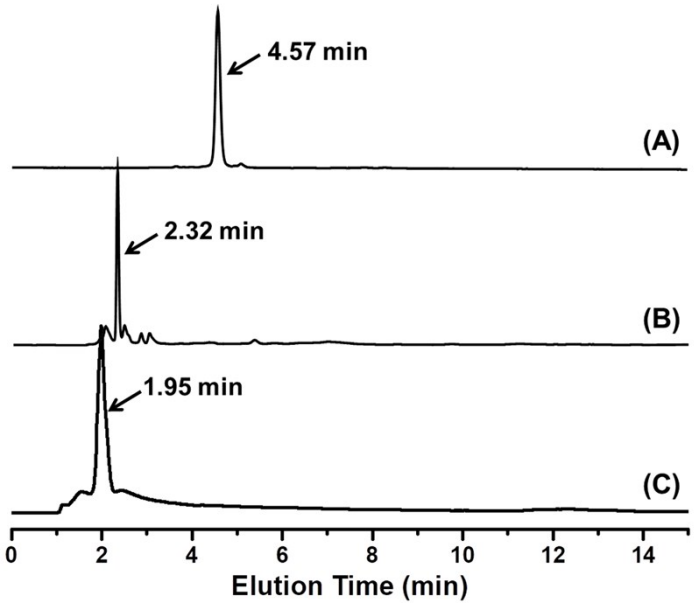

Figure S6. HPLC analyses results of (A) DOX, (B) DOX-*hyd*-N_3 and (C) mPEG-*b*-PBYP-*hyd*-DOX; HPLC analyses were performed with acetonitrile-water (50/50, *v*/*v*) as the mobile phase at 30 °C with a flow rate of 1.0 mL min^{-1}.

Table S2. The drug contents of mPEG-*b*-PBYP-*hyd*-DOX.

Entry	Theory mole ratio mPEG-*b*-PBYP : DOX-N_3	Theory DOX content [a] (*wt*%)	DOX content [b] (*wt*%)
1	1:5	17.50%	13.65%
2	1:15	36.42%	38.32%
3	1:10	28.63%	40.91%
4	1:18	45.48%	36.91%
5	1:20	36.26%	37.01%
6	1:20	36.26%	39.15%

[a] Calculated by the actual addition of DOX-*hyd*-N_3

[b] Calculated by C_{DOX} (%)=($C_{UV\text{-}vis}$/$C_{mPEG\text{-}b\text{-}PBYP\text{-}hyd\text{-}DOX}$)×100, where $C_{UV\text{-}vis}$ represents the concentration of DOX measured by UV-vis, $C_{mPEG\text{-}b\text{-}PBYP\text{-}hyd\text{-}DOX}$ represents the concentration of prodrug.

Table S3. The molecular weights of mPEG-*b*-PBYP-*g*-DAE.

Entry	$\overline{M}_{n,\ NMR(mPEG\text{-}b\text{-}PBYP\text{-}g\text{-}DAE)}$ [a)] (g mol^{-1})
mPEG-*b*-(PBYP-*g*-DAE)$_{34}$	18000
mPEG-*b*-(PBYP-*g*-DAE)$_{58}$	27200
mPEG-*b*-(PBYP-*g*-DAE)$_{26}$	14900
mPEG-*b*-(PBYP-*g*-DAE)$_{63}$	29100
mPEG-*b*-(PBYP-*g*-DAE)$_{86}$	37900

[a)] Calculated based by Eqation (3) and (4).

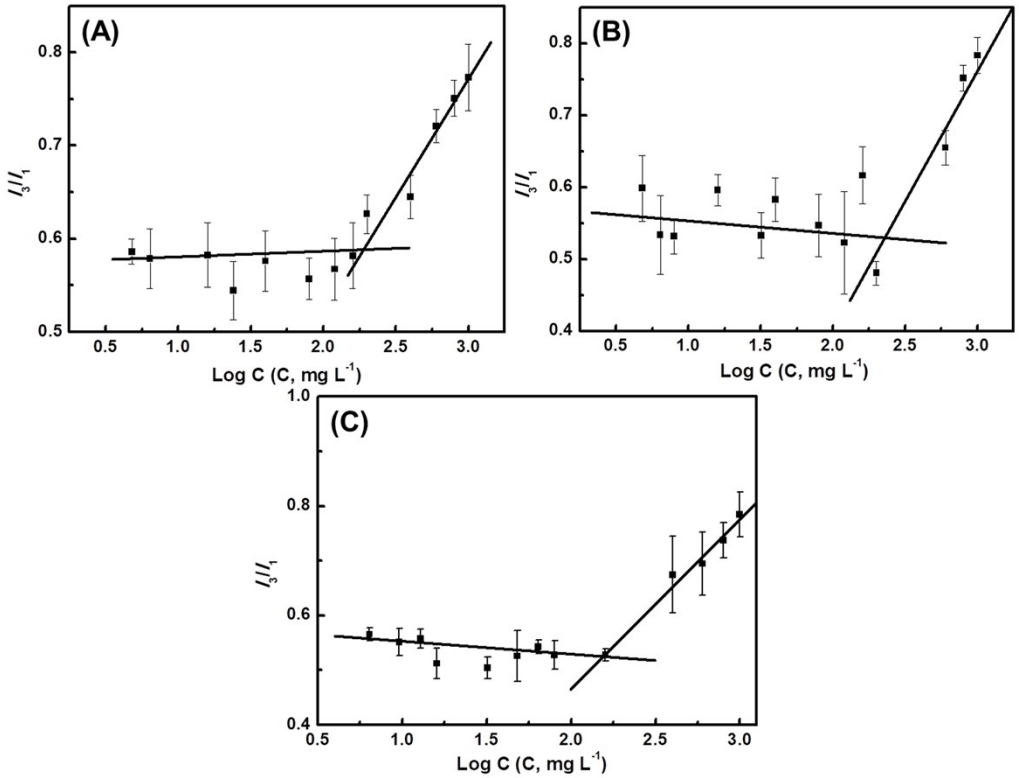

Figure S7. Intensity ratios (I_3/I_1) in fluorescence emission spectra of pyrene as a function of logarithm concentration for different micelles of (A) mPEG-*b*-PBYP-*hyd*-DOX (M-1), (B) mPEG-*b*-PBYP-*g*-DAE (M-2) and (C) hybrid micelles (M-3) in aqueous solution.

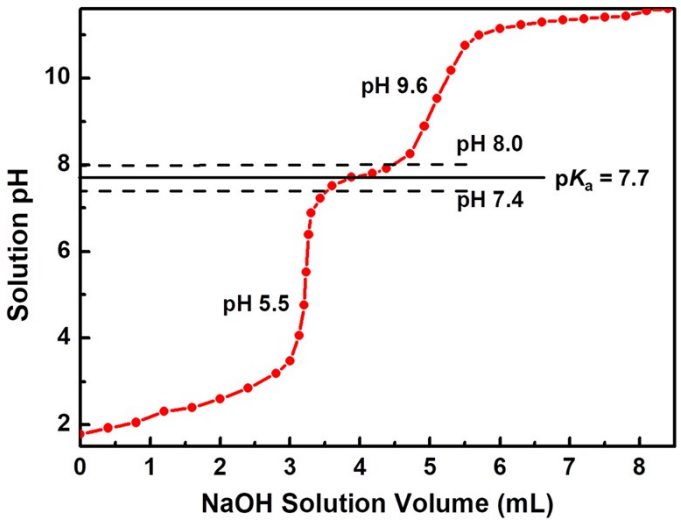

Figure S8. Acid-base titration curves of mPEG-*b*-PBYP-*g*-DAE (1 mg mL^{-1}, from pH 2 to pH 11.5 with 0.02M NaOH) [S4]

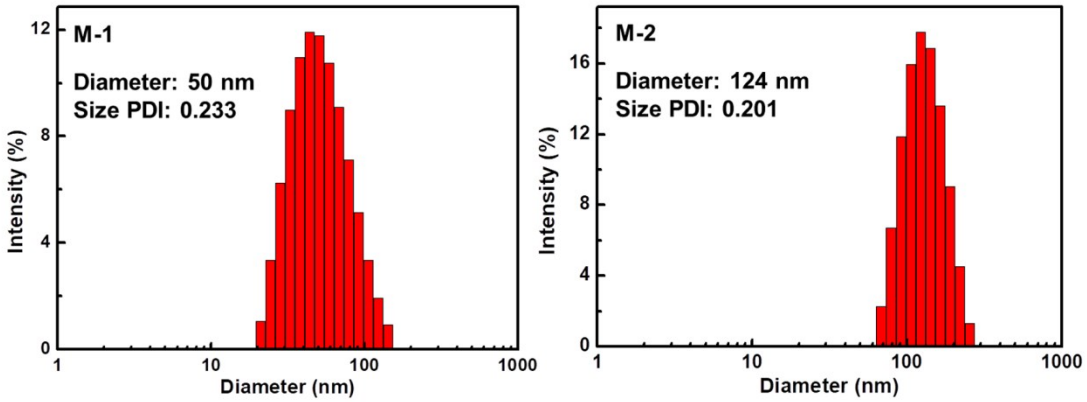

Figure S9. The histogram for the particle size distribution of M-1 and M-2 micelles.

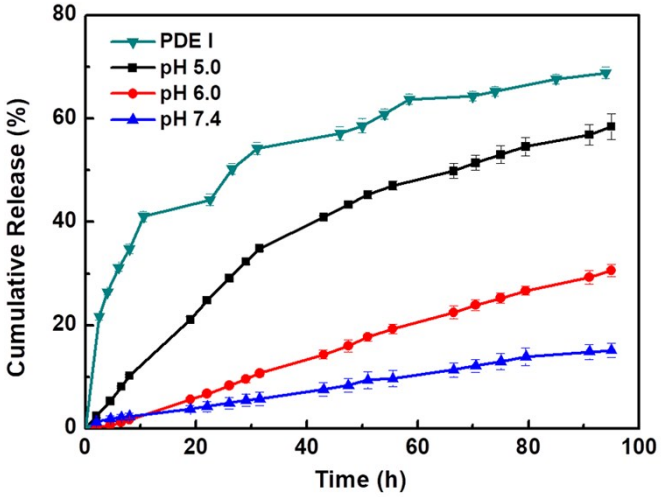

Figure S10. *In vitro* DOX release profiles of M-3 micelles at PB solution and 37.5 °C under different condition.

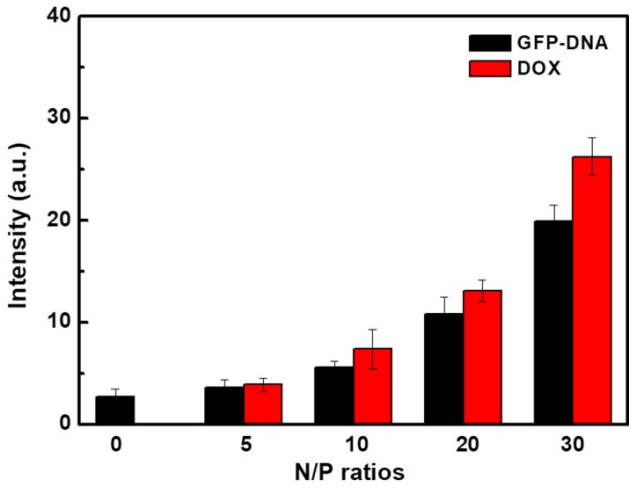

Figure S11. Quantitative determination of fluorescence intensity for A549 cells that were incubated with hybrid micelles/GFP-DNA complexes and free DOX and GFP-DNA at different N/P ratios.

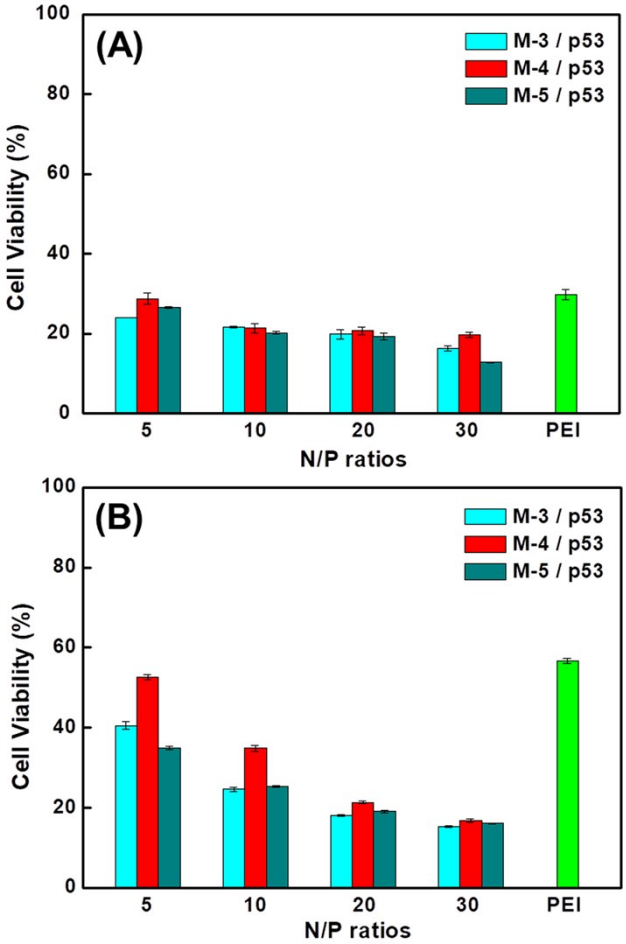

Figure S12. Cell viability of (A) A549 cells and (B) H1299 cells treated with different micelles at various N/P ratios for 48 h incubation.

5. References

S1. X. J. Chen, S. S. Parelkar, E. Henchey, S. Schneider and T. Emrick, *Bioconjugate Chem.,* 2012, **23**, 1753-1763.

S2. Y. H. Lim, G. S. Heo, S. Cho and K. L. Wooley, *ACS Macro Lett.,* 2013, **2***,* 785-789.

S3. Y. H. Lim, G. S. Heo, Y. H. Rezenom, S. Pollack, J. E. Raymond, M. Elsabahy and K. L. Wooley, *Macromolecules,* 2014, **47**, 4634-4644.

S4. J. Han, K. Y. Zhou, X. C. Zhu, Q. P. Yu, Y. Ding, X. H. Lu and Y. L. Cai, *Macromol. Rapid Commun.,* 2016, **37**, 1275-1281.