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Self-assembly of Cytotoxic Peptide Conjugated Poly(β-amino ester)s for Synergistic Cancer Chemotherapy

Zeng-Ying Qiao,[a,§] Chun-Yuan Hou,[a,b,§] Di Zhang,[a] Ya Liu,[c] Yao-Xin Lin,[a] Hong-Wei An,[a] Xiao-Jun Li,[b] and Hao Wang*[,a]

[a] CAS Key Laboratory for Biological Effects of Nanomaterials and Nanosafety, National Center for Nanoscience and Technology (NCNST), Beijing, 100190, China.

Email: wanghao@nanoctr.cn

[b] School of Chemical Engineering & Technology, Hebei University of Technology, Tianjin, 300130, China.

[c] College of Marine Life Science, Ocean University of China, No. 5 Yushan Road, Qingdao, China.

§ These authors contributed equally to this work.

Particle Size Measurements

The hydrodynamic diameters and particle size distributions of the copolymer micelles were measured on a dynamic light scattering (DLS) analyzer (Zetasizer Nano ZS). The micelle dispersion (1 mg/mL, pH 7.4 PB) was passed through syringe filters (0.45μm, Millipore) before measurements. The measurements of the copolymer micelles (1 mg/mL) in buffer solutions (10 mM PB, pH 7.4 or 50 mM acetate buffer, pH 5.0) were performed at 25 ^{o}C.

The morphologies of copolymer micelles were observed by transmission electron microscopy (TEM, Tecnai G2 20 S-TWIN) with an acceleration voltage of 200 kV. The copolymer micelle dispersions (0.5 mg/mL) in PB (pH 7.4, 10 mM) were formed by dialysis method. The micelle dispersions (10 μL) were dropped onto a copper mesh, and then most of the liquid was dried by a filter paper after 2 min. The samples were stained with 10 μL of uranyl acetate solution for 40 s followed by drying the spare liquid with the filter. Finally, 10 μL of deionized water was used to wash the copper mesh, which was blotted after 30 s and dried at room temperature.

Circular Dichroism (CD) Spectra

Free KLAK (0.27 mg/mL), **P2** micelles (0.73 mg/mL), **P2**-KLAK micelles (1 mg/mL) were prepared by dialysis method, and the CD spectra were recorded using a circular dichroism spectrometer (JASCO-1500, Japan) with a cell path length of 1 mm at room temperature. The measurements were performed at a scanning speed of 1000 nm minutes^{-1} and a resolution of 0.5 nm. Ten spectra were accumulated and averaged for each sample. The fraction of secondary structure elements of the free KLAK and conjugated KLAK were estimated from the respective CD spectra using standard CONTINLL algorithms.

Nile Red (NR) Fluorescence

The encapsulation stability and pH-dependent dissociation profiles of the copolymer micelles were measured using hydrophobic NR as fluorescent probe. NR in ethanol (12 μL, 1.0 x 10^{-3} mol/L) was added into 6 mL of the micelle dispersion (1.0 mg/mL, pH 7.4, 10 mM PB). The final concentration of NR was 2.0×10^{-6} mol/L. The micelle dispersions were stirred overnight in dark at room temperature, and then the measurements were performed on an F-280 fluorometer with the excitation wavelength of 545 nm. The first obtained data in pH 7.4 buffer were used as that for 0 time point. For the fluorescent measurement of dissociation profiles, the pH of micelle dispersion was adjusted to 5.0 by adding acetate buffer (pH 5.0, 5.0 M), and then the fluorescence spectra of the dispersions were measured at different time intervals.

Stability of DOX-loaded P2-KLAK Micelles

The DOX-loaded **P2**-KLAK micelles were prepared as above mentioned in phosphate buffer saline (PBS, 10 mM, pH 7.4). The hydrodynamic diameters of the copolymer micelles (1.0 mg/mL) in PBS solutions were monitored at 37 ^{o}C. The measurements were operated every 30 min to obtain the diameter changes of DOX-loaded **P2**-KLAK micelles.

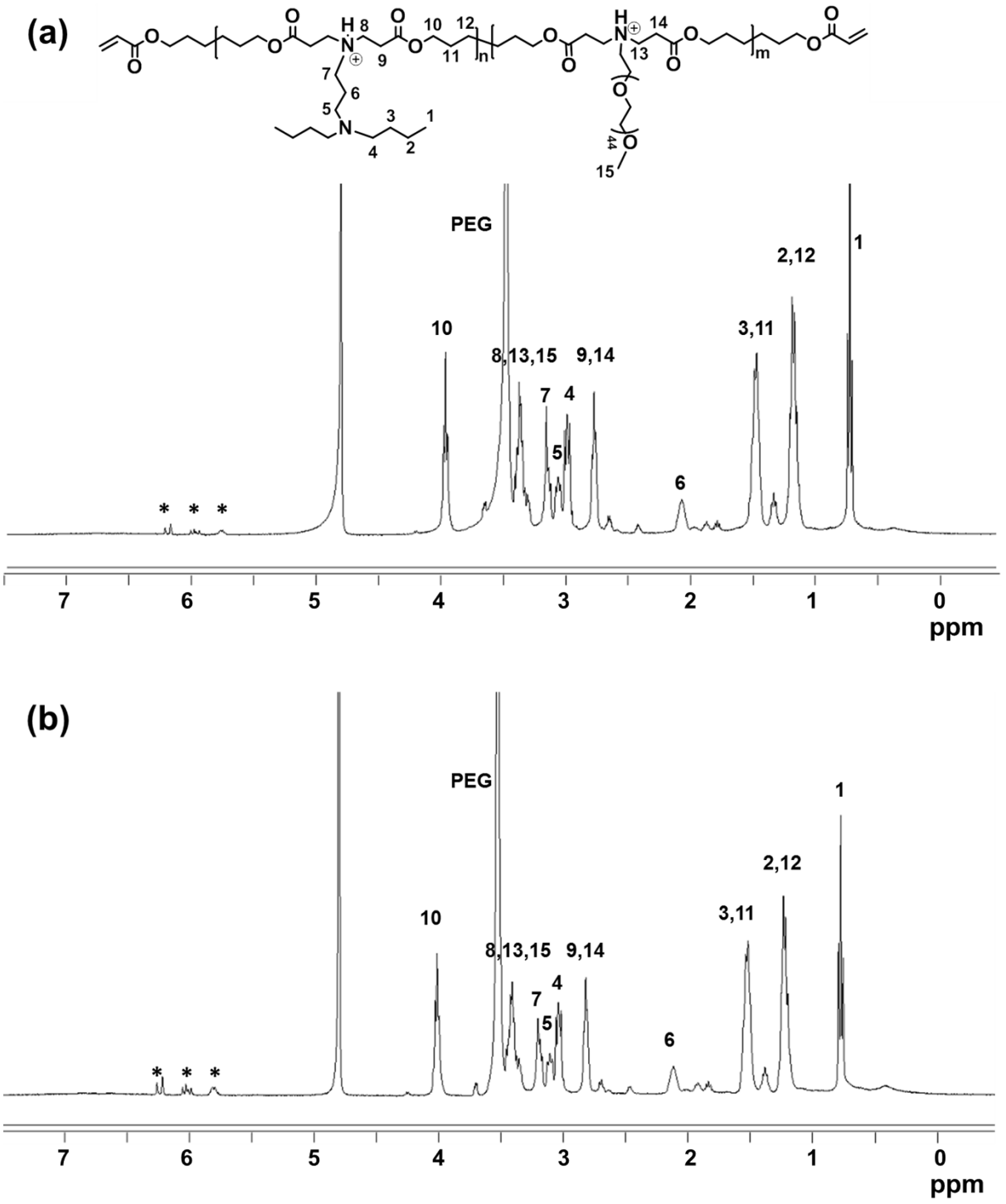


Fig. S1. ^{1}H NMR spectra of poly(β-amino ester)s copolymers **P1** (a) and **P3** (b) in D_2O containing 0.6 wt% DCl. Asterisks (*) represent the double bonds of acrylate end groups.

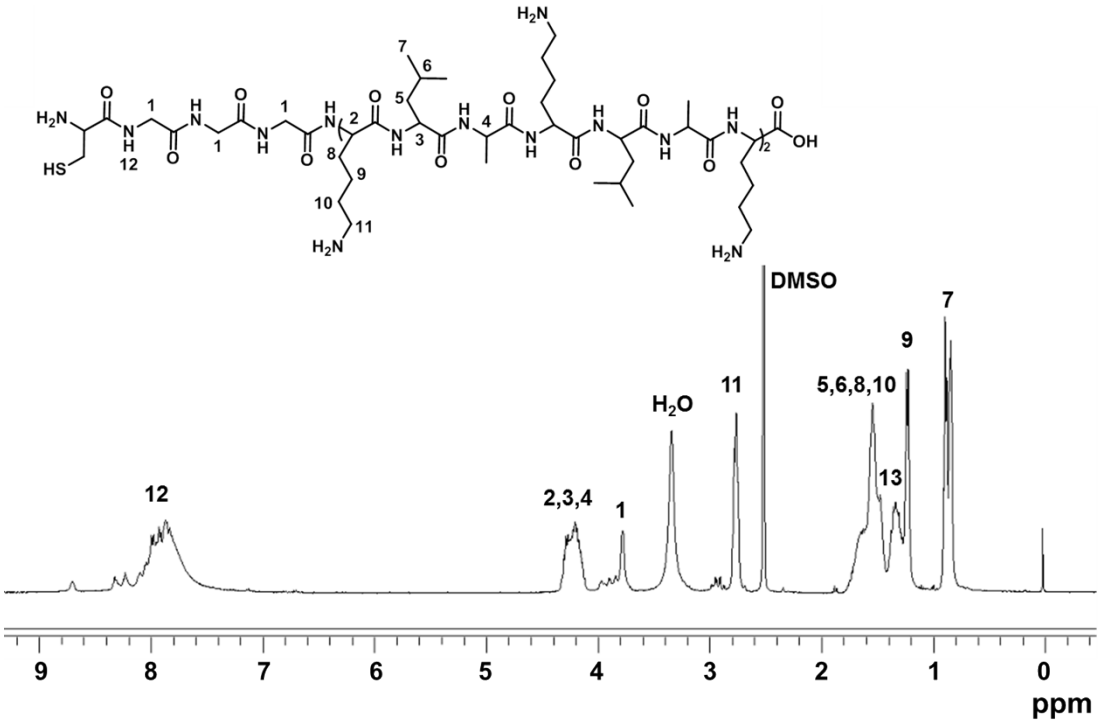


Fig. S2. ^{1}H NMR spectrum of KLAK peptide in d^{6}-DMSO.

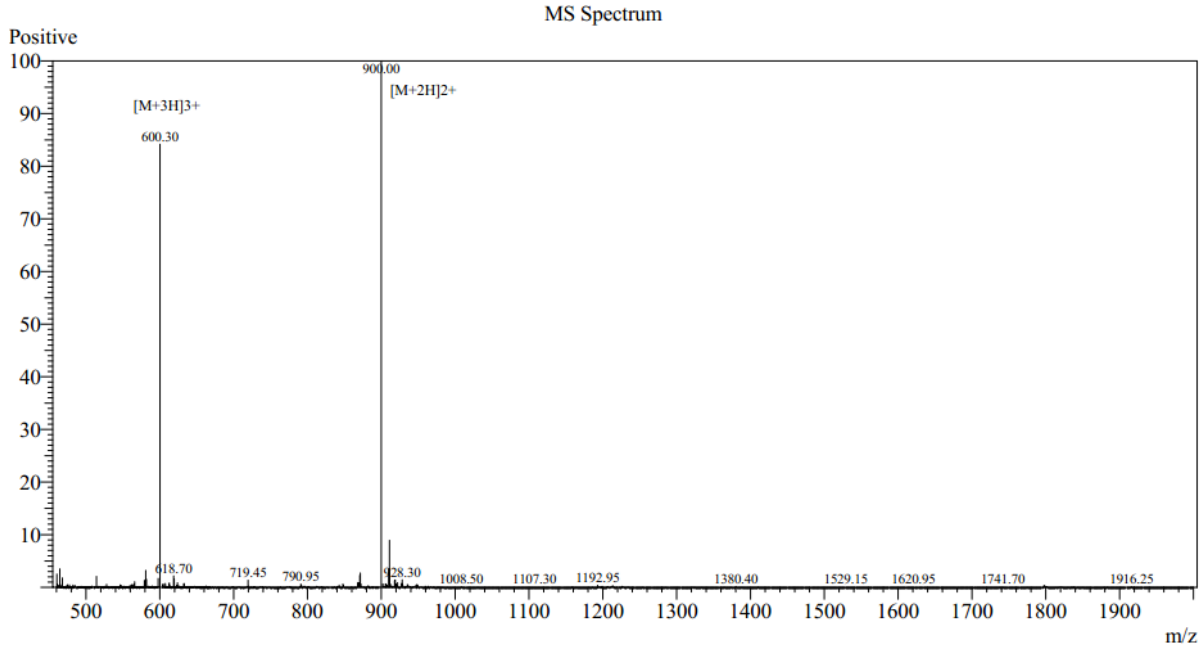
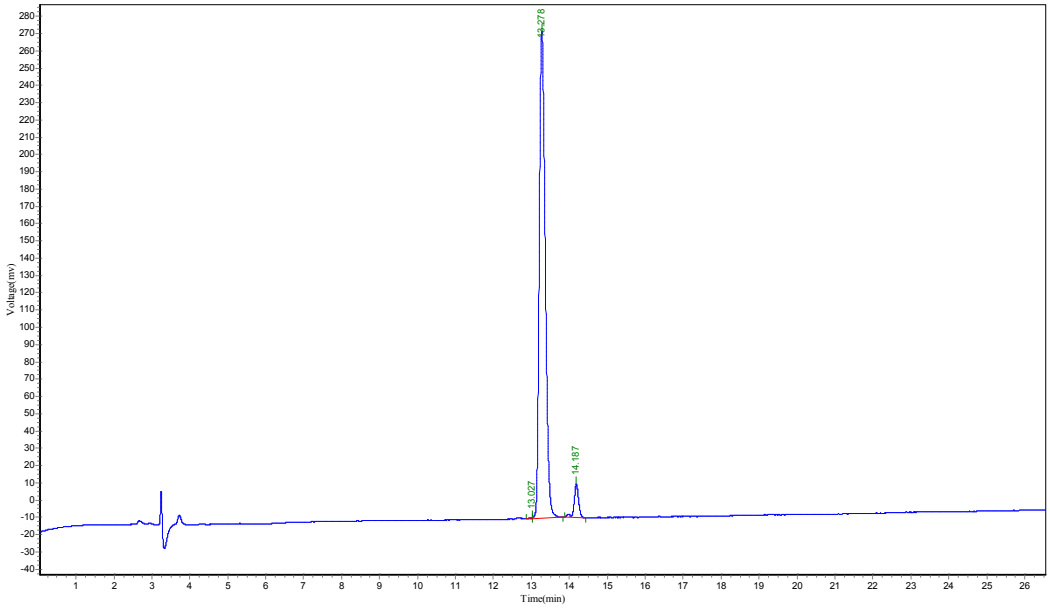


Fig. S3. The MS spectrum of peptide CGGG(KLAKLAK)$_2$.



Peak No	Ret Time	Height	Area	Conc.
1	13.027	400.013	1841.090	0.0569
2	13.278	282180.281	3076027.750	95.0821
3	14.187	19289.199	157259.563	4.8610
				100.00

Fig. S4. The HPLC spectrum of peptide CGGG(KLAKLAK)$_2$.

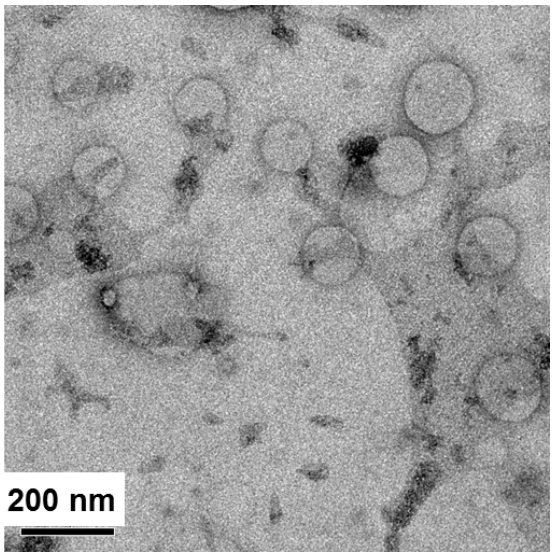


Fig. S5. TEM images of and **P2** in aqueous solutions at pH 5.0.

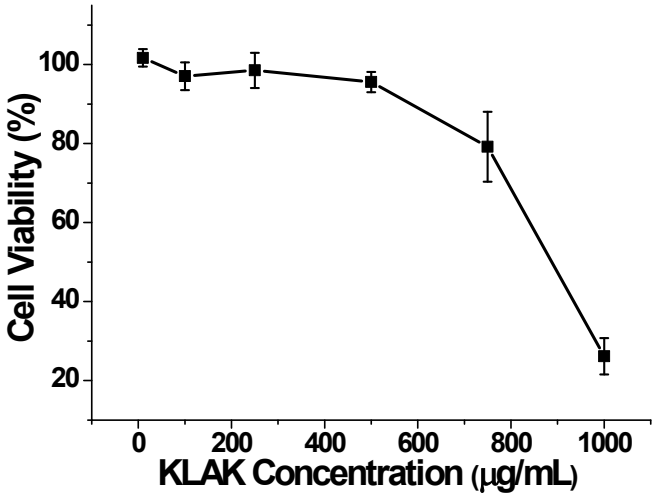


Fig. S6. MCF-7 cell viability incubated with KLAK at various concentration measured by the CCK-8 assay. Results are presented as the mean±SD in triplicate.

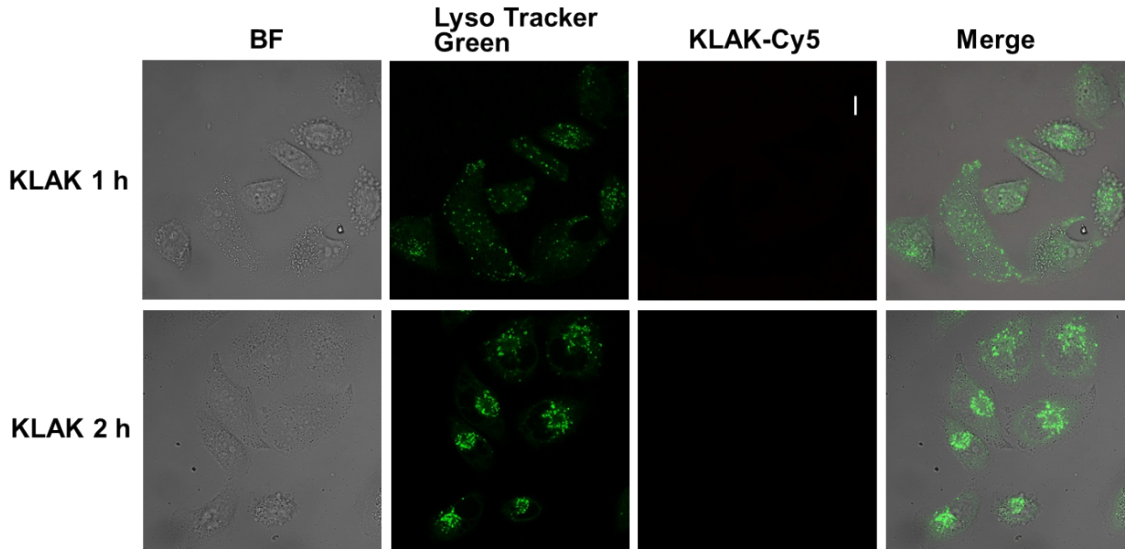


Fig. S7. CLSM microscopy of living MCF-7 cells that were incubated with KLAK peptide for 1 h and 2 h. KLAK concentration: 45 μg/mL. Lysosomes were labeled with LysoTracker Green DND-26 for 30 min before imaging.

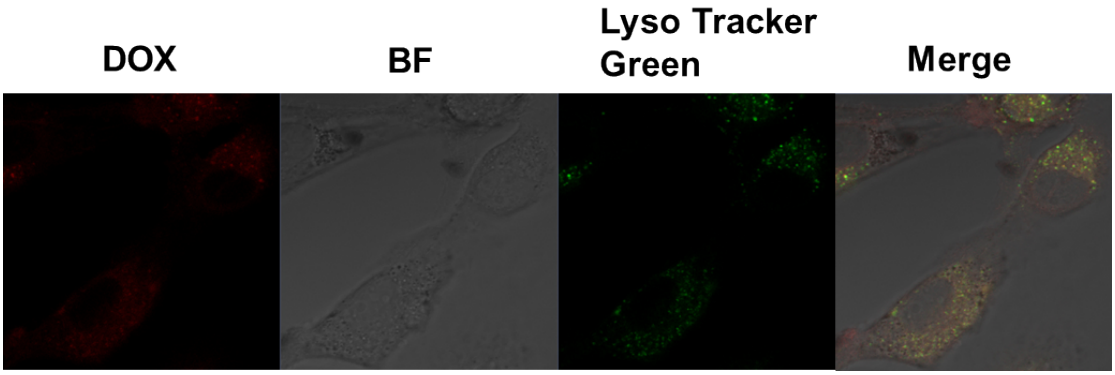


Fig. S8. CLSM microscopy of living MCF-7 cells that were incubated with DOX-loaded **P2**-KLAK micelles for 1 h. DOX concentration: 5 μg/mL. Lysosomes were labeled with LysoTracker Green DND-26 for 30 min before imaging.

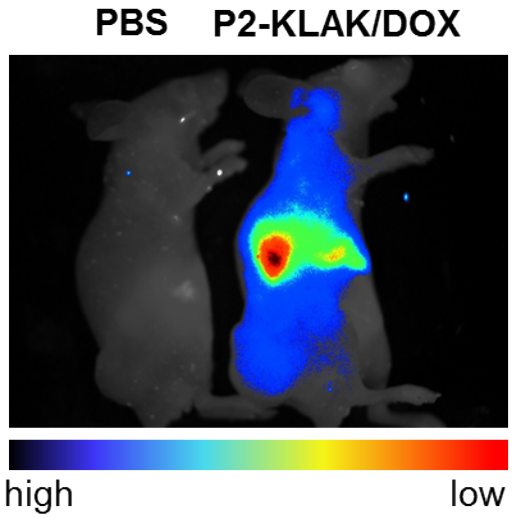


Fig. S9. *In vivo* NIR optical imaging (Ex. 680 nm, Em. 726 nm) of nude mice bearing MCF-7 tumors 4 h post tail-vein injection of **P2**-KLAK/DOX (right). Mice treated with PBS were regarded as the control group (left).