Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.

Supporting Information

Improving the Carrier Stability and Drug Loading of Unimolecular Micelle-based Nanotherapeutic for Acid-actived Drug Delivery and Enhanced Antitumor Therapy

Xiaoxiao Shi[a, b], *Shuang Bai*[a, b], Cangjie Yang[c], *Xiaoqian Ma*[a, b], *Meili Hou*[a, b], *Jiucun Chen*[a, b], *Peng Xue*[a, b], *Chang Ming Li*[a, b], *Yuejun Kang*[a, b]*, *Zhigang Xu*[a, b]*

[a]Institute for Clean Energy and Advanced Materials, Faculty of Materials and Energy, Southwest University, Chongqing 400715, P. R. China

[b]Chongqing Engineering Research Centre for Micro-Nano Biomedical Materials and Devices, Chongqing 400715, P.R. China.

[c]School of Chemical and Biomedical Engineering, Nanyang Technological University, 62 Nanyang Drive, Singapore 637459, Singapore

*Corresponding Authors

Z. Xu (zgxu@swu.edu.cn); Fax: +86-23-68253204; Tel: +86-23-68253792

Y. Kang (yjkang@swu.edu.cn); Fax: +86-23-68253204; Tel: +86-23-68254056

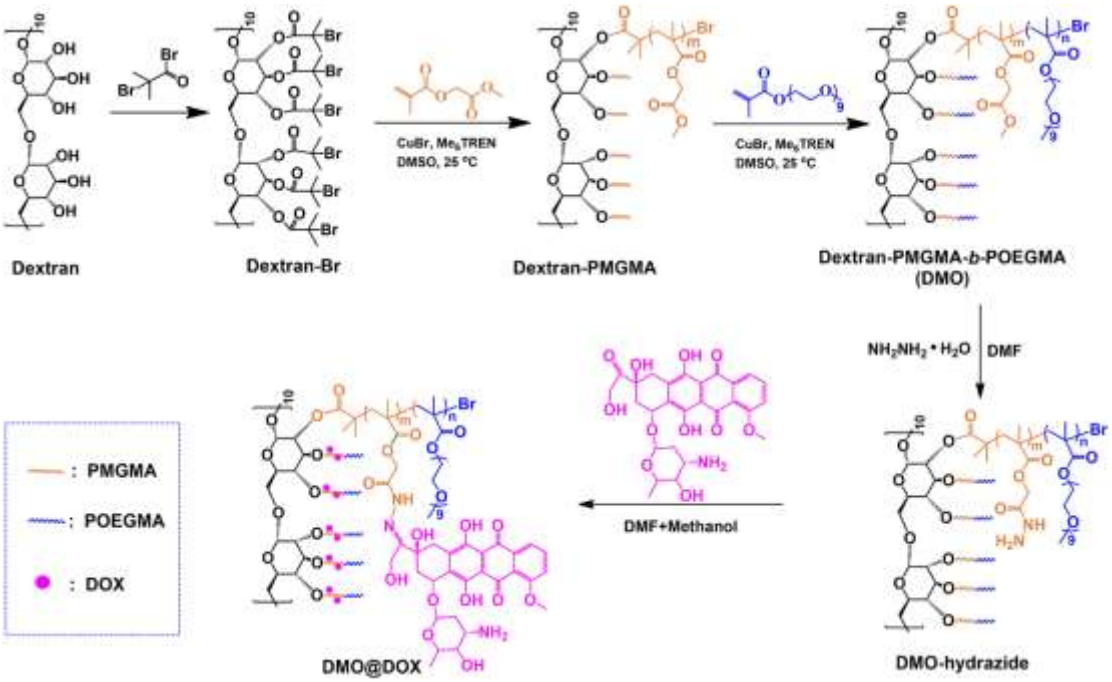

Figure S1. The synthetic route of rod-like DMO@DOX polymeric prodrug.

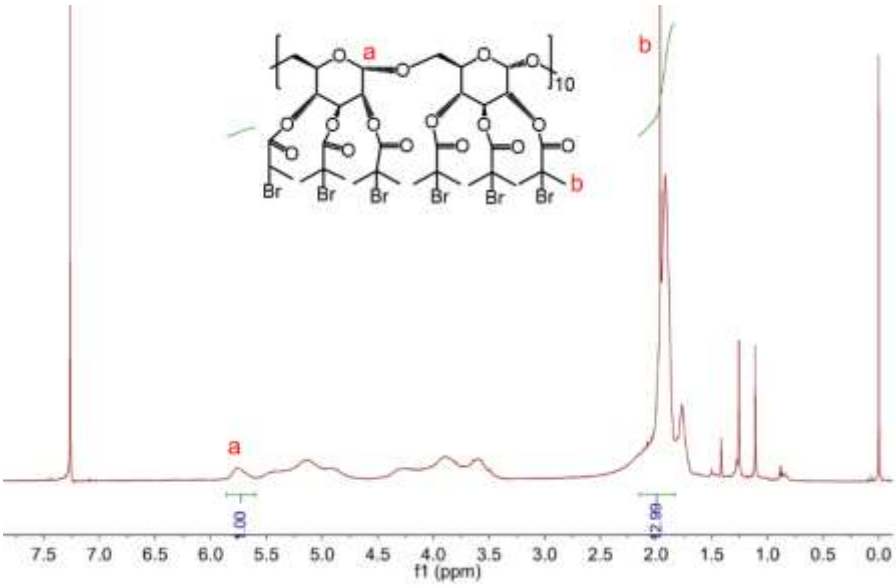

Figure S2. The ^{1}H NMR spectrum of DEX-Br macroinitiator.

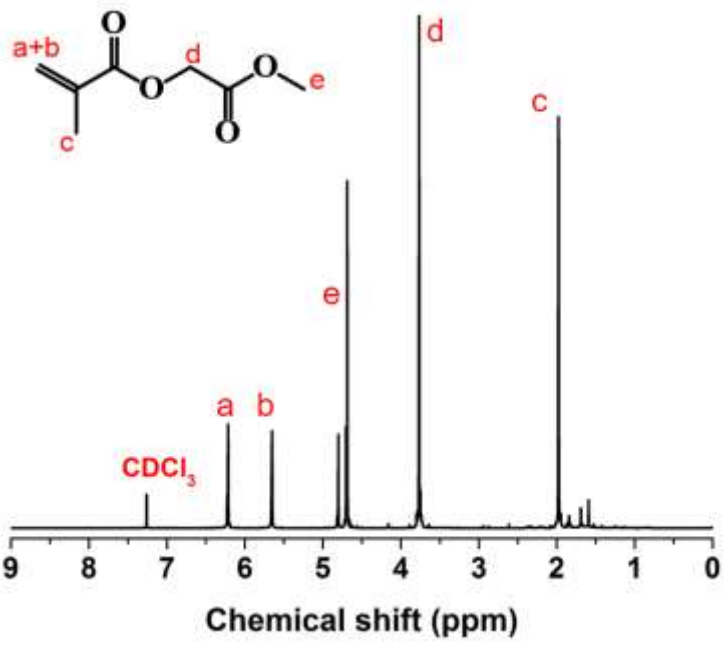

Figure S3. The ^{1}H NMR spectrum of MGMA monomer.

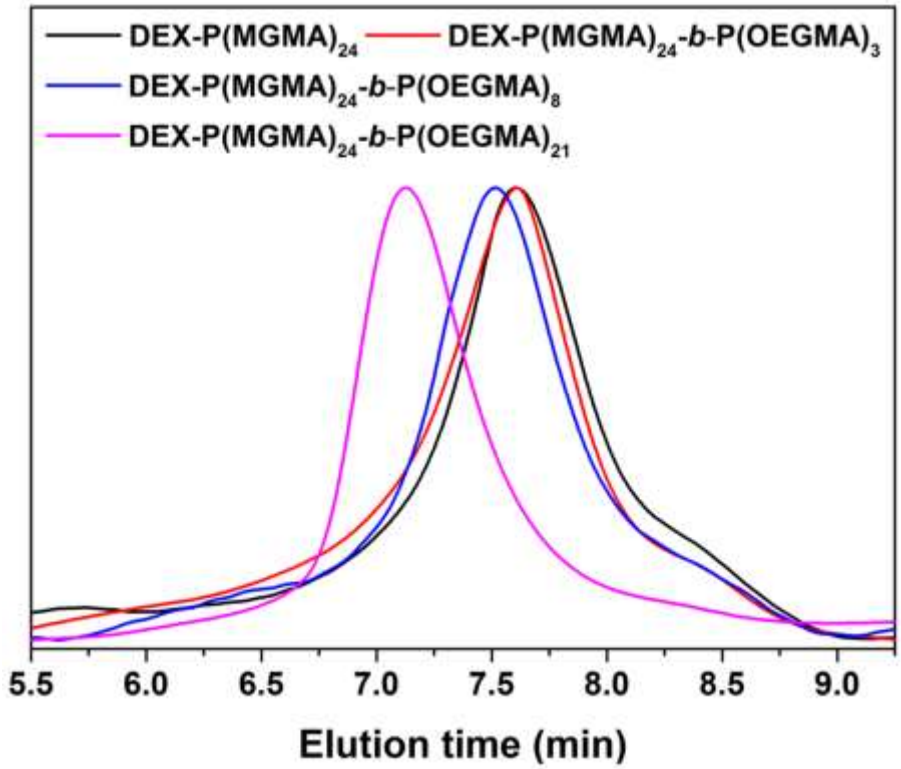

Figure S4. GPC traces of DEX-PMGMA$_{24}$ and DEX-P(MGMA)$_{24}$-*b*-P(OEGMA)$_3$ (DMO-1), DEX-P(MGMA)$_{24}$-*b*-P(OEGMA)$_8$ (DMO-2) and DEX-P(MGMA)$_{24}$-*b*-P(OEGMA)$_{21}$ (DMO-3).

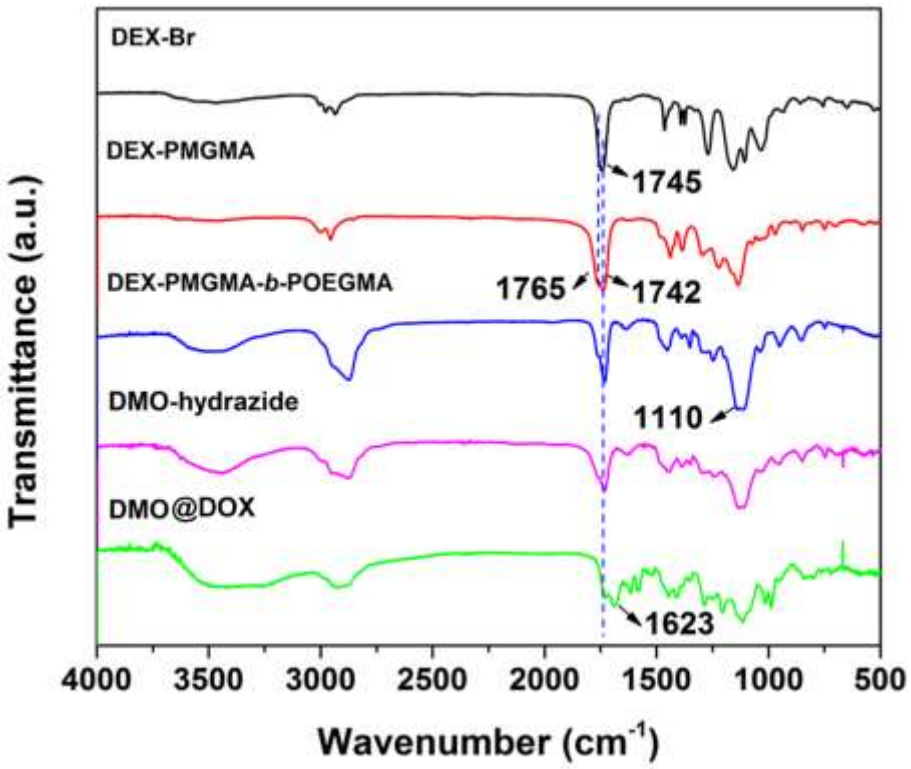

Figure S5. Typical FTIR spectra of DEX-Br, DEX-PMGMA, DEX-PMGMA-*b*-POEGMA (DMO), DMO-hydrazide and DMO@DOX, respectively.

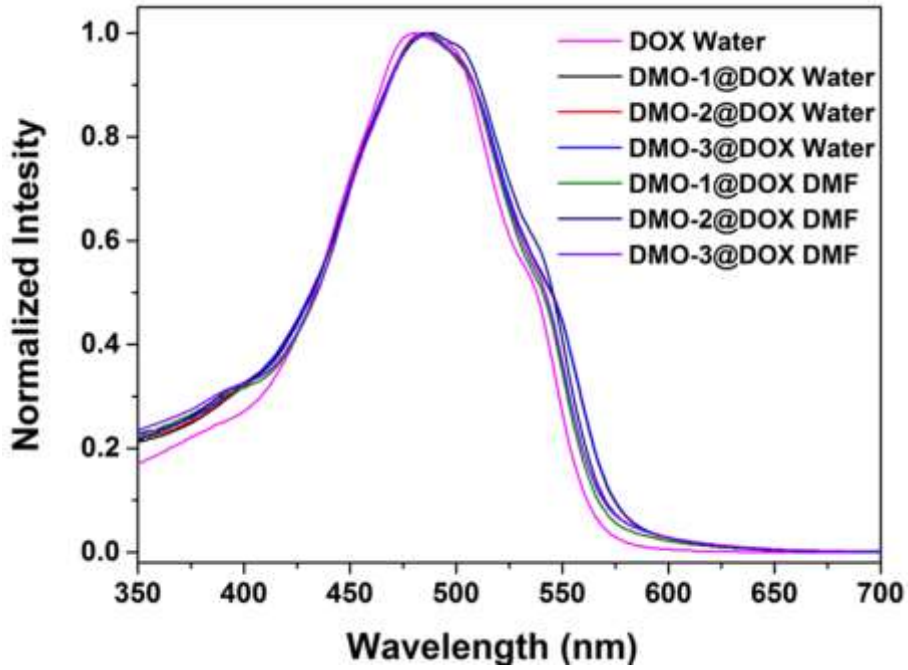

Figure S6. UV-vis spectra of free DOX, DMO-1@DOX, DMO-2@DOX and DMO-3@DOX prodrugs in water or DMF solution.

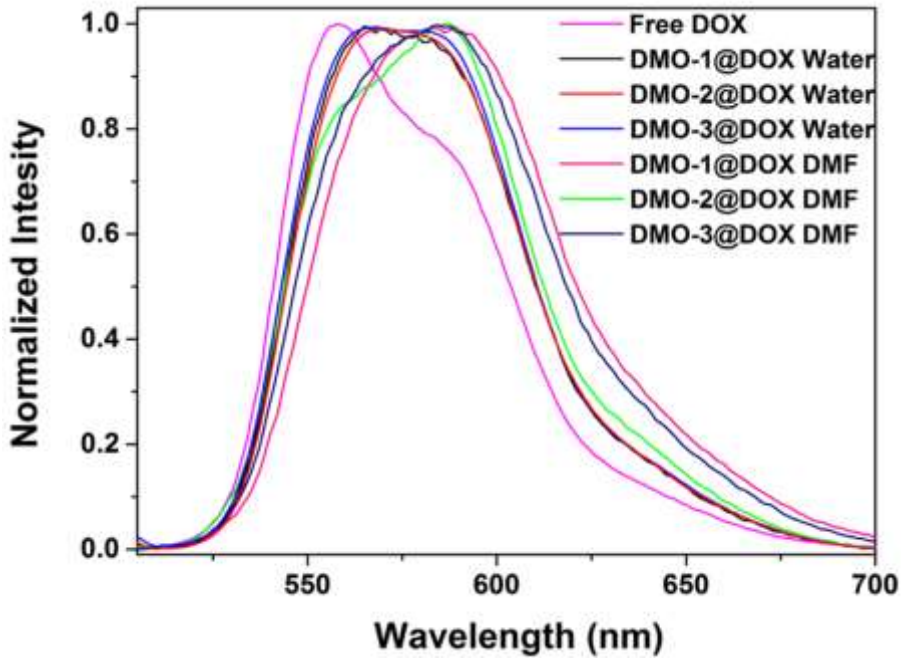

Figure S7. The fluorescence spectra of free DOX, DMO-1@DOX, DMO-2@DOX and DMO-3@DOX prodrugs in water or DMF solution.

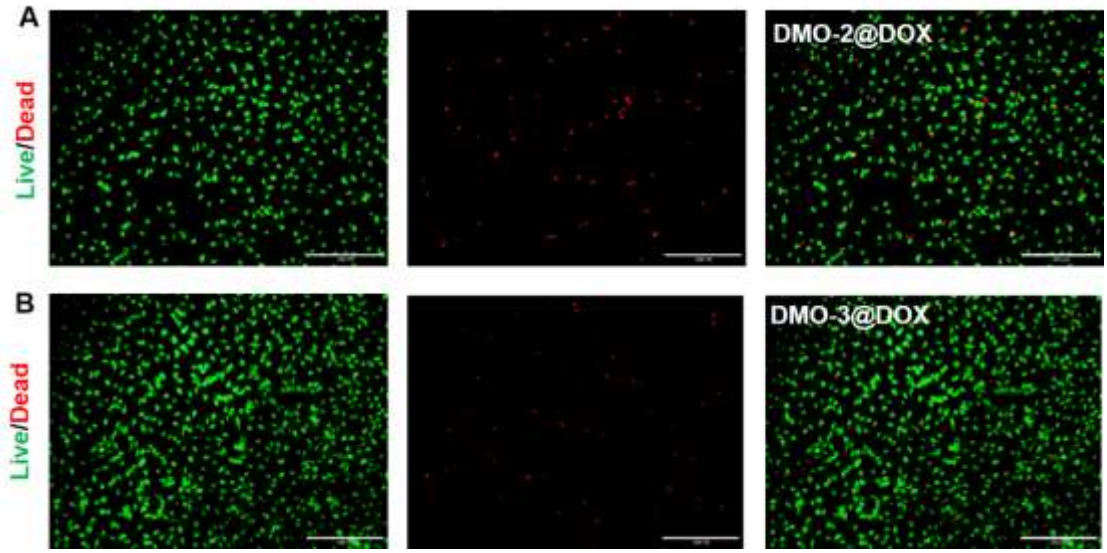

Figure S8. Fluorescence images of HeLa cells incubated with (A) DMO-2@DOX UMs and (B) DMO-3@DOX UMs for 24 h, respectively (final equivalent DOX concentration: 15 μg/mL). The fluorescence of Calcein AM (live cells) and PI (dead cells) were labelled as green and red, respectively. Scale bars: 200 μm.

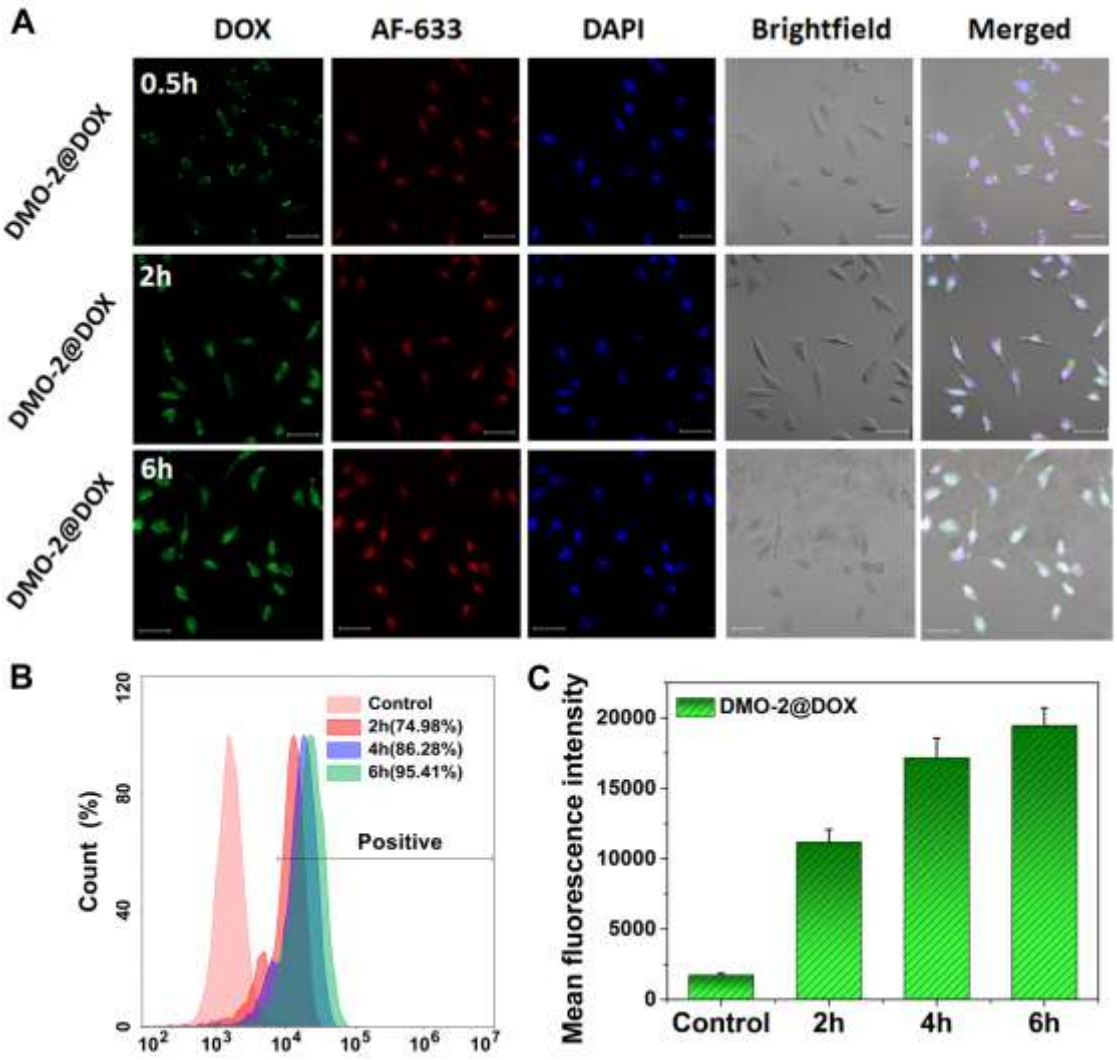

Figure S9. (A) CLSM images showing the distribution of free DOX and DMO-2@DOX UMs in HeLa cells (final equivalent DOX concentration: 20 μg/mL). Cell nuclei and cytoplasm were stained with DAPI (Blue) and AF-633 (Red), respectively. Scale bars: 50 μm. (B) Flow cytometry analysis and (C) the mean fluorescence intensity of DMO-2@DOX treated HeLa cells at 2, 4 and 6 h (data are presented as means ± SD, n =3).

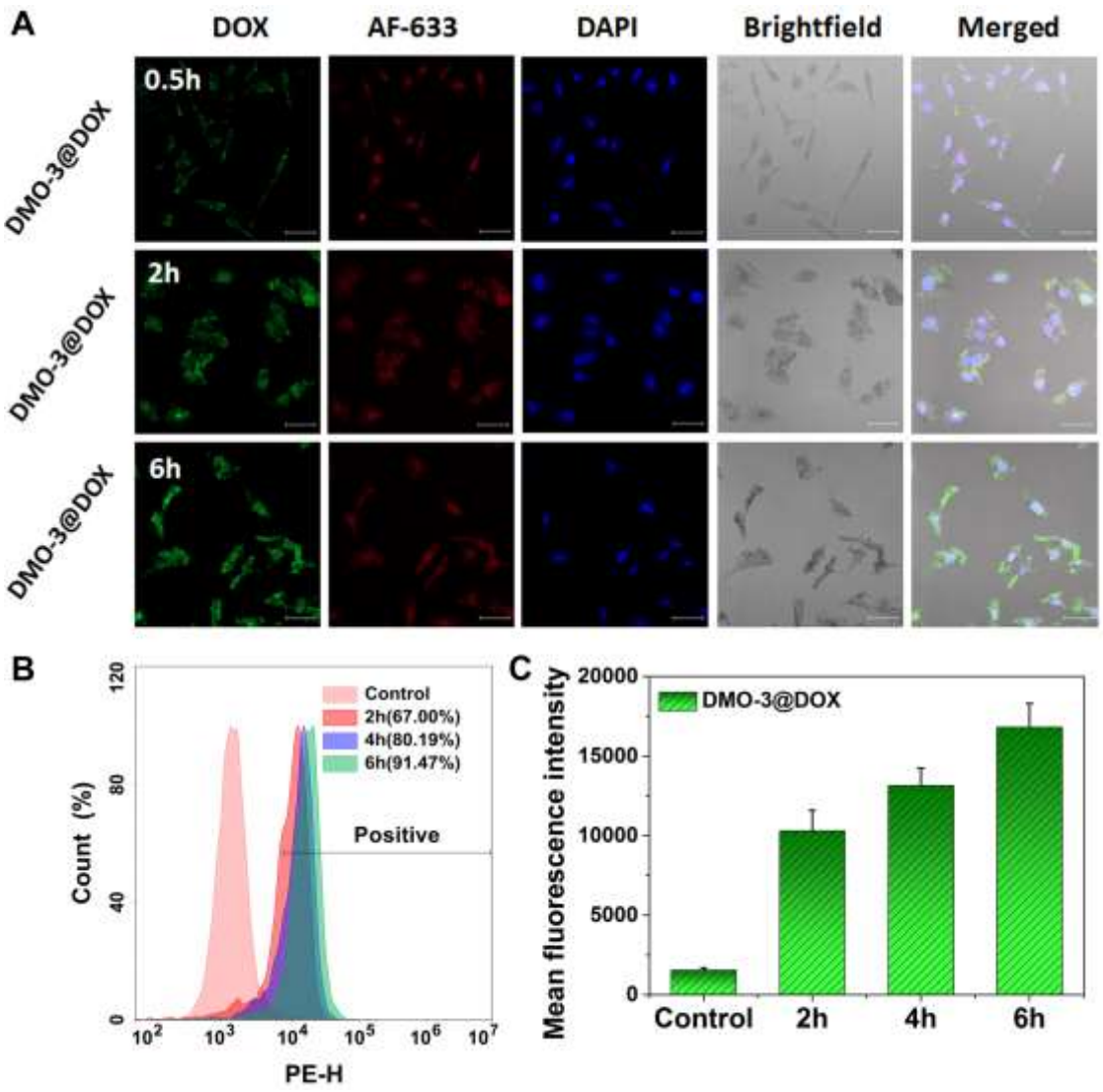

Figure S10 (A) CLSM images showing the distribution of free DOX and DMO-3@DOX UMs in HeLa cells (final equivalent DOX concentration: 20 μg/mL). Cell nuclei and cytoplasm were stained with DAPI (Blue) and AF-633 (Red), respectively. Scale bars: 50 μm. (B) Flow cytometry analysis and (C) the mean fluorescence intensity of DMO-3@DOX treated HeLa cells at 2, 4 and 6 h (data are presented as means ± SD, n =3).

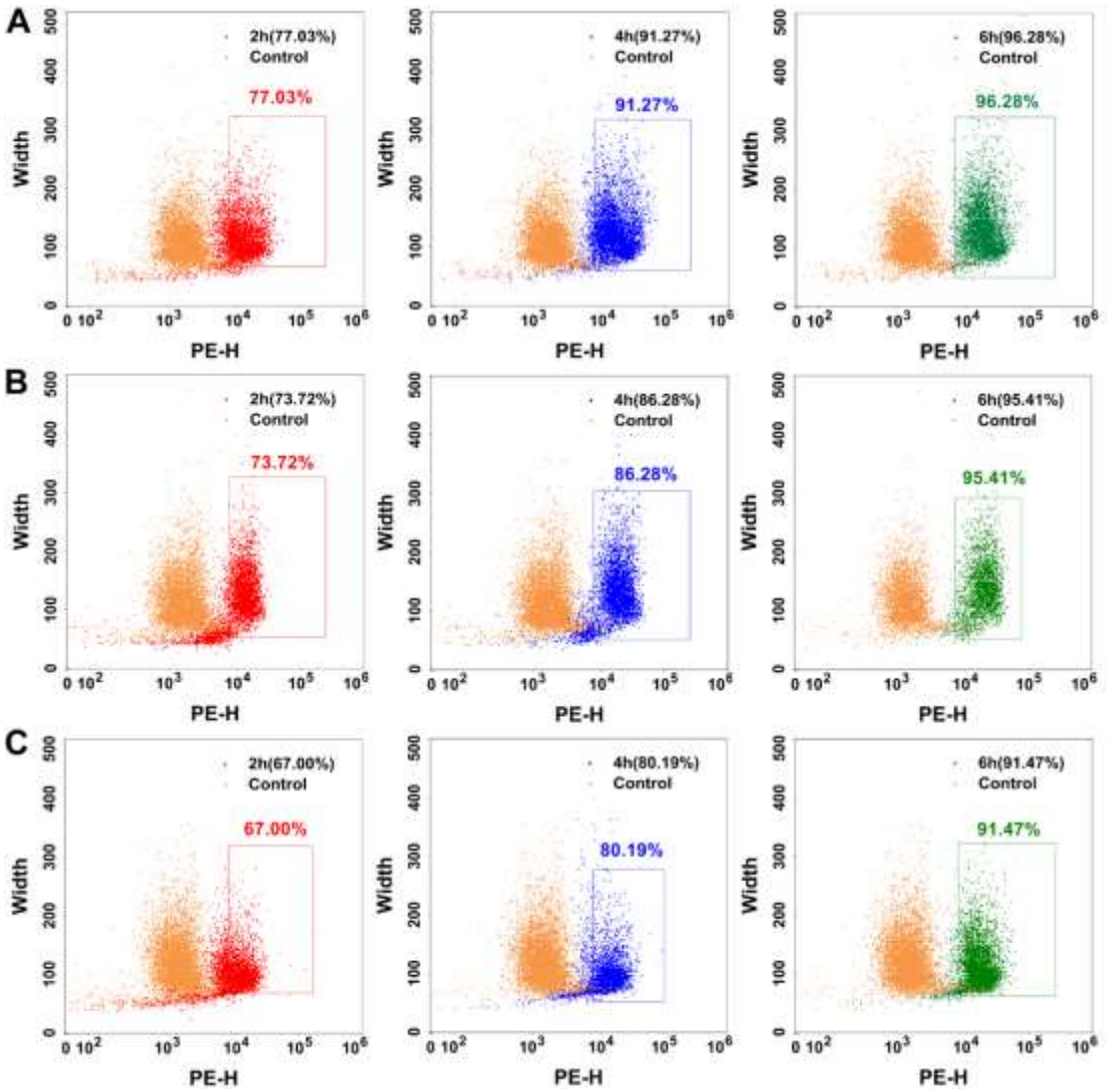

Figure S11 Flow cytometry dot plots for HeLa cells treated with (A) DMO-@DOX, (B) DMO-2@DOX and (C)DMO-3@DOX UMs under different incubation time.

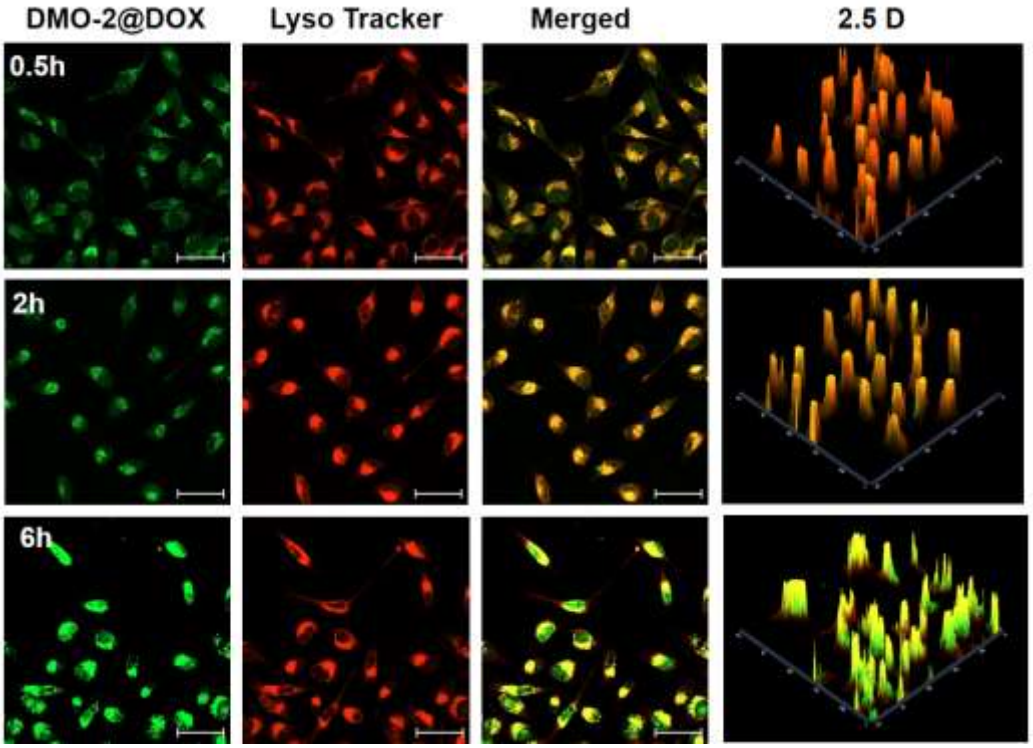

Figure S12 CLSM images showing the time-dependent accumulation process of DMO-2@DOX UMs using Lyso Tracker as the tracer (final equivalent DOX concentration: 20 μg/mL). Scale bars: 50 μm.

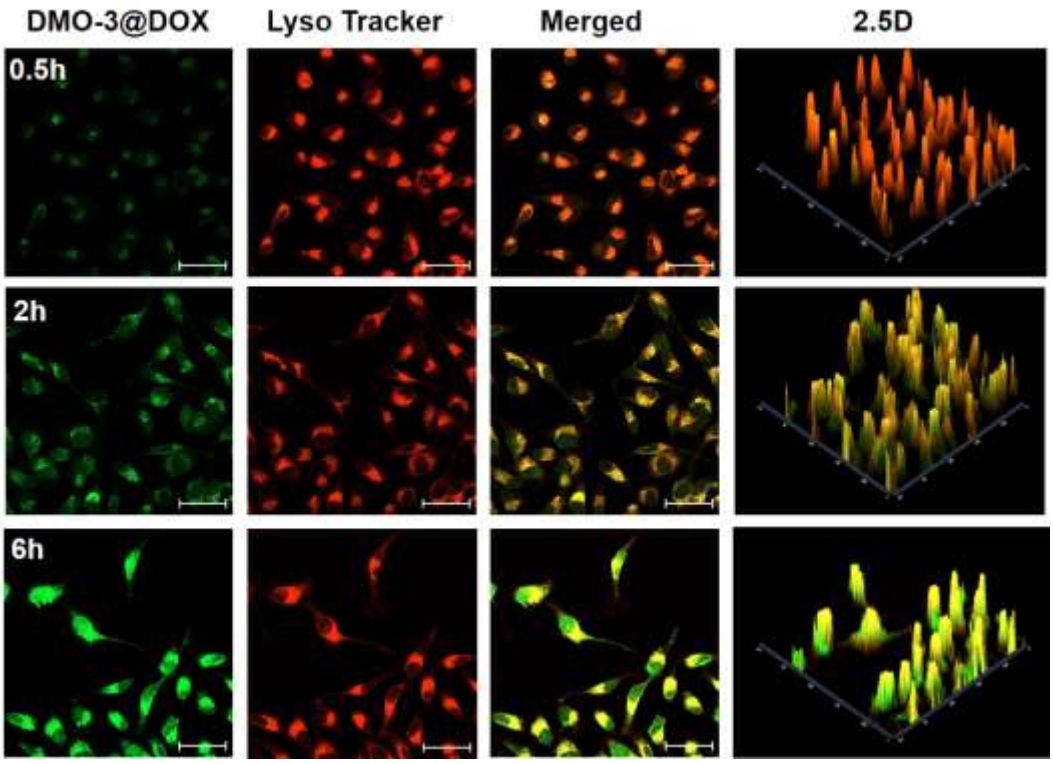

Figure S13 CLSM images showing the time-dependent accumulation process of DMO-2@DOX UMs using Lyso Tracker as the tracer (final equivalent DOX concentration: 20 μg/mL). Scale bars: 50 μm.

Table S1. Structural information of the rod-like DMO@DOX prodrug and their precursors

Polymer [a]	$M_{n,NMR}$ [b] (g mol^{-1})	$M_{n,GPC}$ [c] (g mol^{-1})	M_w/M_n [d]	D^e (Water)	D^f (DMF)	LC^g (wt%)
DEX-P(MGMA)$_{24}$	252900	38900	1.46	—	—	—
DEX-P(MGMA)$_{24}$-*b*-P(OEGMA)$_3$	339200	42900	1.56	34.83	45.89	80.4
DEX-P(MGMA)$_{24}$-*b*-P(OEGMA)$_8$	496900	48500	1.28	41.35	55.96	72.2
DEX-P(MGMA)$_{24}$-*b*-P(OEGMA)$_{21}$	912200	95500	1.24	68.01	74.23	40.4

[a] DP_n of polymerized alternating units (MGMA or OEGMA) and [b]$M_{n,NMR}$ was measured by ^{1}H NMR results. [c]$M_{n,GPC}$ and [d]M_w/M_n were measured by GPC. [e]Hydrodynamic diameter of prodrug micelles were determined by DLS. [g]The loading content (LC) were determined by Fluorescence (FL) spectrophotometer, respectively.

Table S2.IC_{50} value of free DOX and DMO@DOX prodrug. [a]

Sample	IC_{50}(HeLa) μg/ml	IC_{50}(MCF-7) μg/ml	IC_{50}(HUVEC) μg/ml
Free DOX	0.072	0.06	0.075
DMO-1@DOX	1.61	0.77	>10
DMO-2@DOX	0.65	1.75	>10
DMO-3@DOX	0.83	1.91	>10

a: IC_{50} value of free DOX, DMO-1@DOX, DMO-2@DOX and DMO-3@DOX determined in HeLa cells, MCF-7 cells and HUVEC cells by PrestoBlue assay, and cells were incubated with samples for 72 h.