Electronic supplementary information (ESI)

An autonomous tumor-targeted nanoprodrug for reactive oxygen species-activatable dual-cytochrome c/doxorubicin antitumor therapy

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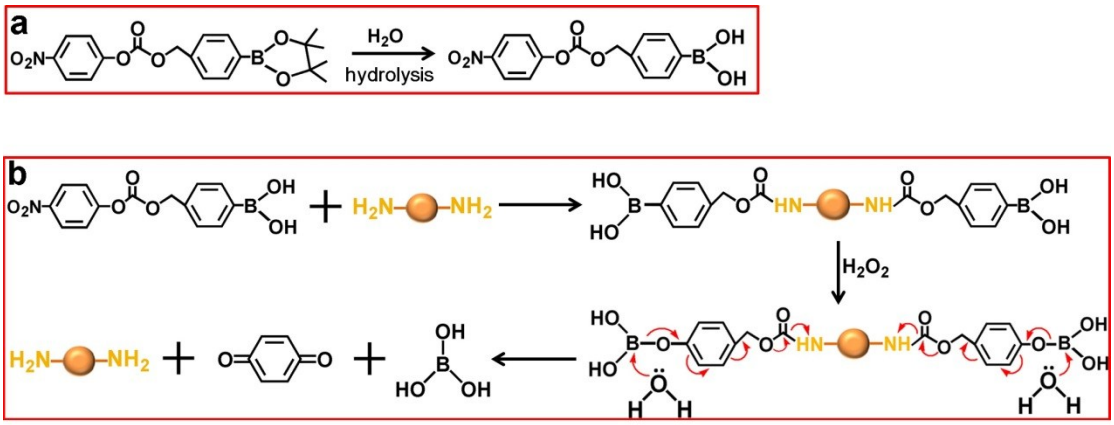


Scheme S1. a) Synthesis of 4-nitrophenyl 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl) benzyl carbonate(NBC) via hydrolysis of boronic acid within water and b) Modification scheme of Cytochrome c with NBC for ROS-activatable intracellular protein delivery.[1]

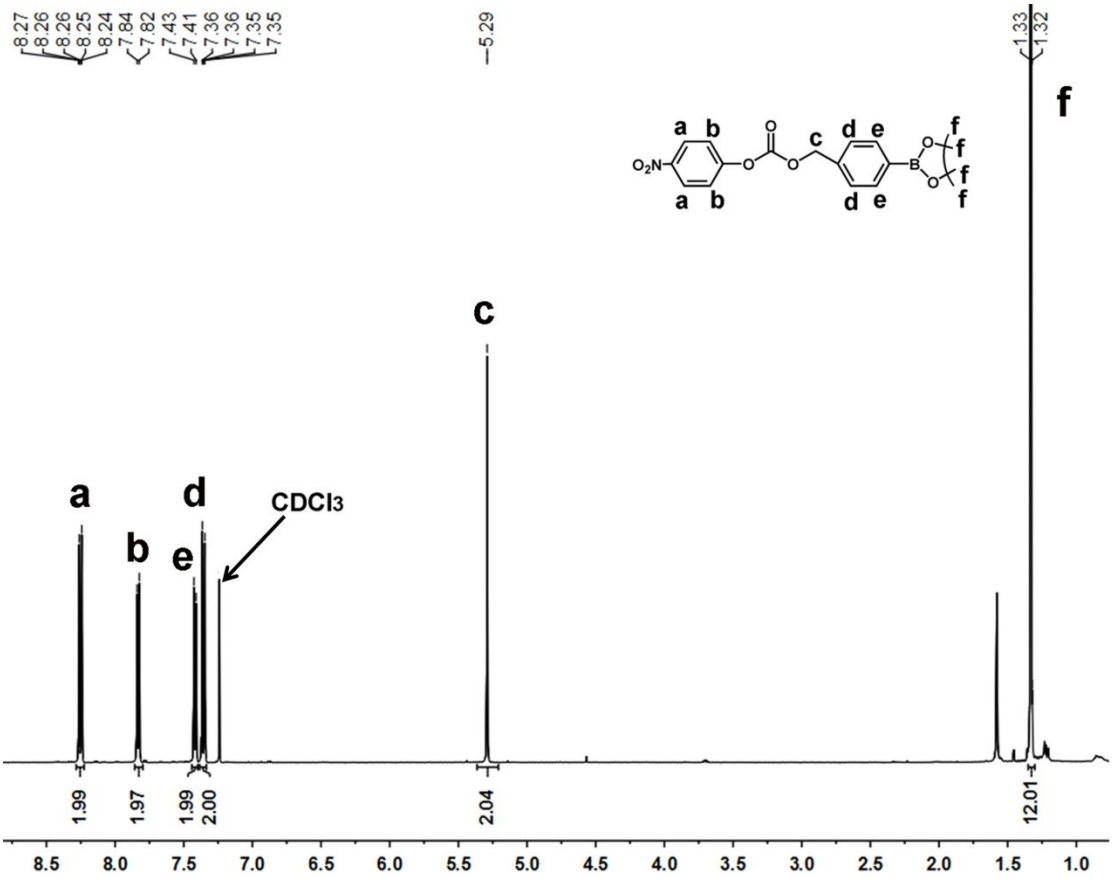
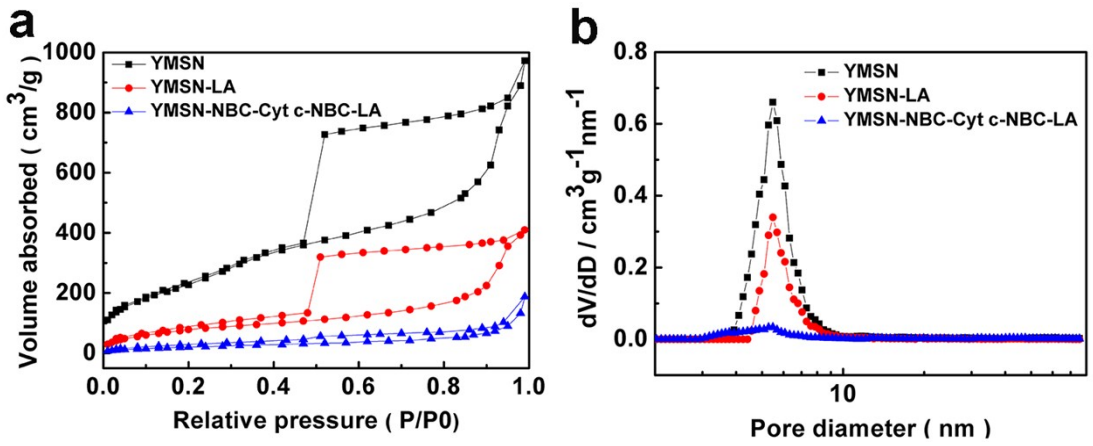


Fig. S1 [1]H NMR spectra (400 MHz, 298 K) of 4-nitrophenyl 4-(4,4,5,5-tetramethyl-1,3,2- dioxaborolan-2-yl)benzyl carbonate.[2]



c

Materials	S_{BET} (m^2/g)	V_P ($cm^3.g^{-1}$)	BJH pore Diameter（nm）
YMSN	917.838	1.3536	4.004
YMSN-LA	403.366	0.5898	4.016
YMSN-NBC-Cyt c-NBC-LA	121.308	0.2284	3.589

Fig. S2 (a) Nitrogen adsorption/desorption isotherms and (b) pore size distributions of different YMSN-based samples determined by BET and BJH, respectively. (c) Porous characteristics of different YMSN-based nanostructures.

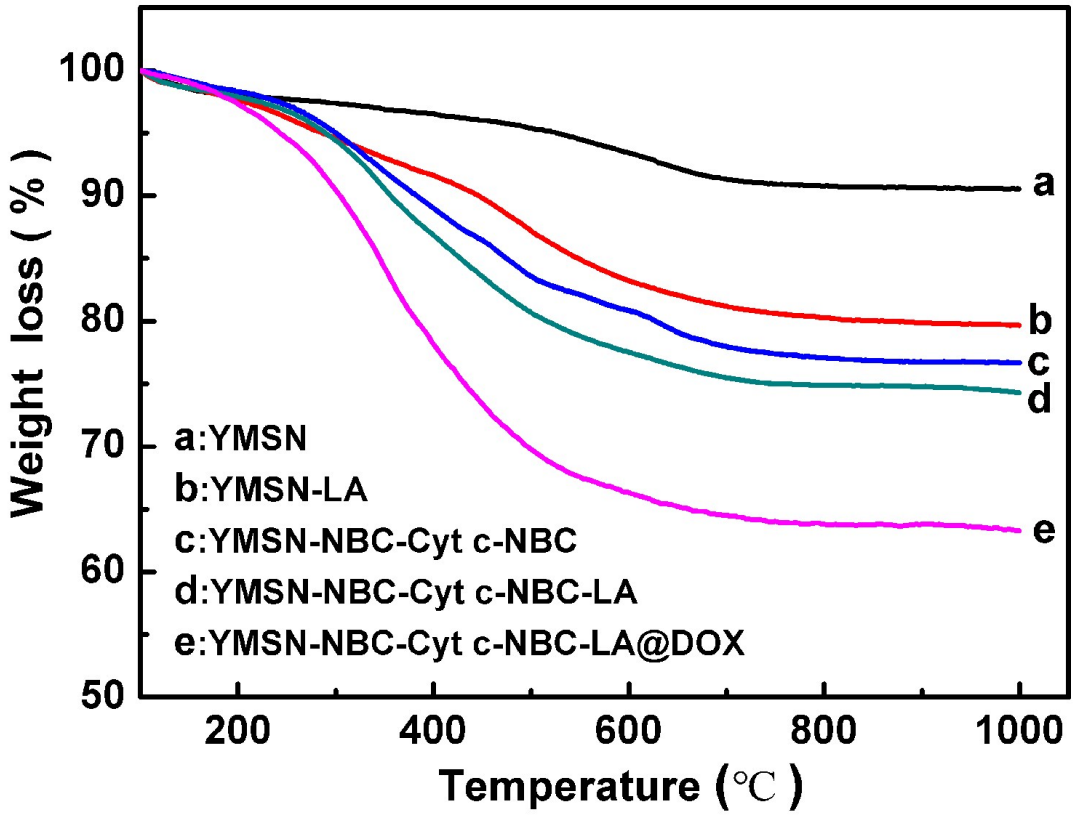


Fig. S3 TGA analysis of YMSN(a), YMSN-LA(b), YMSN-NBC-Cyt c-NBC (c) ,YMSN-NBC-Cyt c-NBC-LA (d) and YMSN-NBC-Cyt c-NBC-LA @DOX (e), respectively.

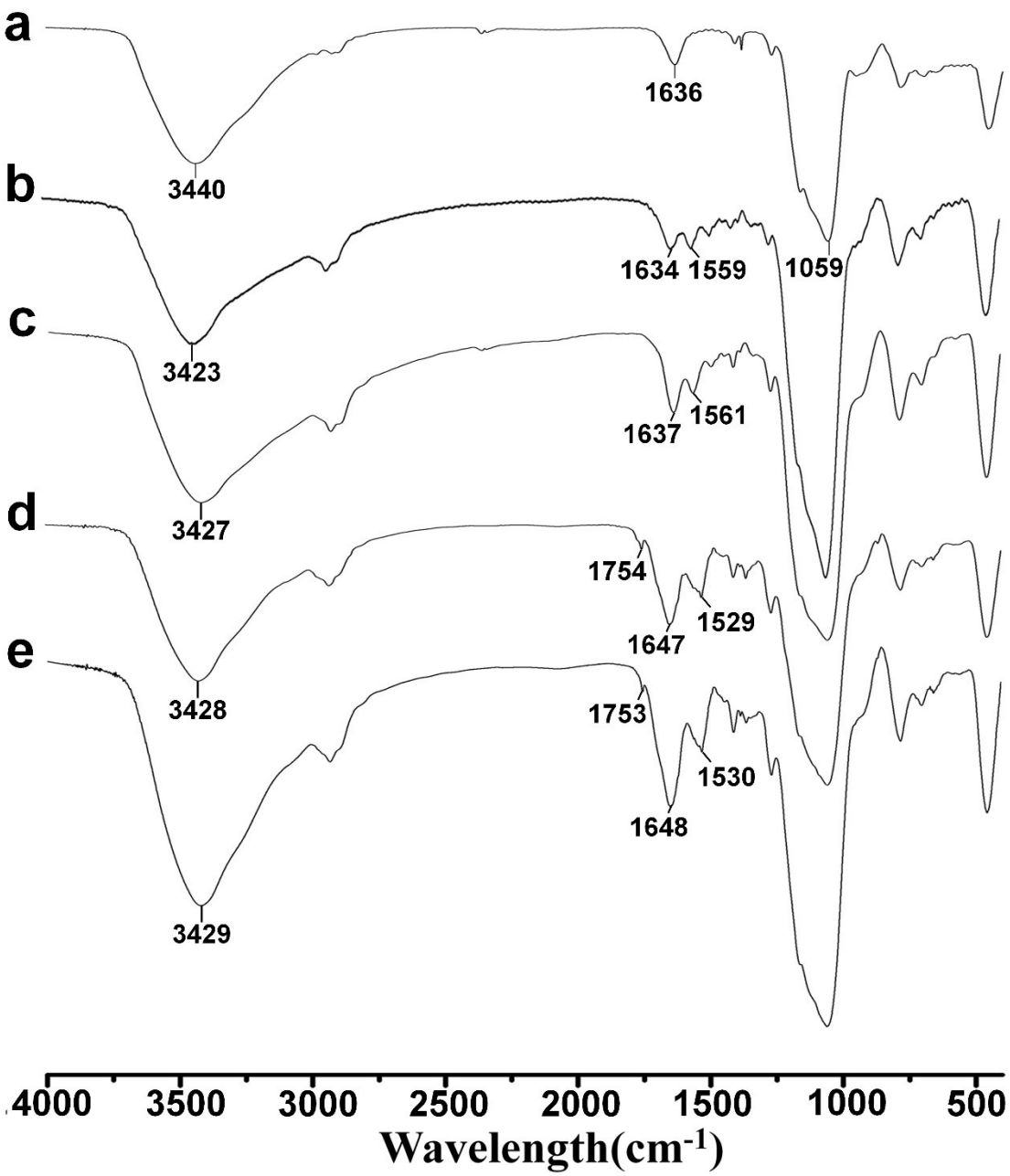


Fig. S4 FTIR spectra of YMSN(a), YMSN-NH_2(b), YMS-LA (c), YMSN-NBC-Cyt c-NBC (d) and YMSN-NBC-Cyt c-NBC-LA (e), respectively. Specifically, YMSNs showed a strong absorption peak at 1059 cm^{-1}, which was due to the asymmetric stretching vibration of the Si-O-Si bonds. The other two peaks at 3440 cm^{-1} and 1636 cm^{-1} were assigned to the Si-OH bonds. After the conjugation of APTES, two new peaks emerged at 1634 cm^{-1} and1559 cm^{-1}, which were due to the Amide I and II vibration modes, respectively. For the IR spectrum of YMSN-LA, it could be observed that the Amide I and II bands have strengthened significantly. Moreover, these two bands have shifted from 1634 cm^{-1} and 1559 cm^{-1} to 1637 cm^{-1} and 1561 cm^{-1}, indicating the successful conjugation of LA units. A new peak at 1754 cm^{-1} has appeared in the IR spectrum of YMSN-NBC-Cyt c-NBC, which was caused by the C=O bonds in the added NBC-Cyt c-NBC units. It could also be observed that Amide

I/II bands for YMSN-NBC-Cyt c-NBC have shifted to 1647 cm^{-1} and 1529 cm^{-1}, respectively, which was caused by the enhanced vibration affected by the -NH- moiety on Cyt c. For the final product of YMSN-NBC-Cyt c-NBC-LA, the intensity of the NBC-associated wide band has decreased due to the conjugation of additional LA units, while the characteristic Amide I/II bands have further shifted to 1648 cm^{-1} and 1530 cm^{-1}. [3]

Table .S1 Zeta potential of the nanoparticulate samples.

Materials	ζ-potential(mV)
YMSN	-26.6±1.39
YMSN-NH_2	23.1±2.1
YMSN-LA	-3.89±1.24
YMSN-NBC-Cyt c-NBC	6.25±0.5
YMSN-NBC-Cyt c-NBC-LA	-11.37±0.5

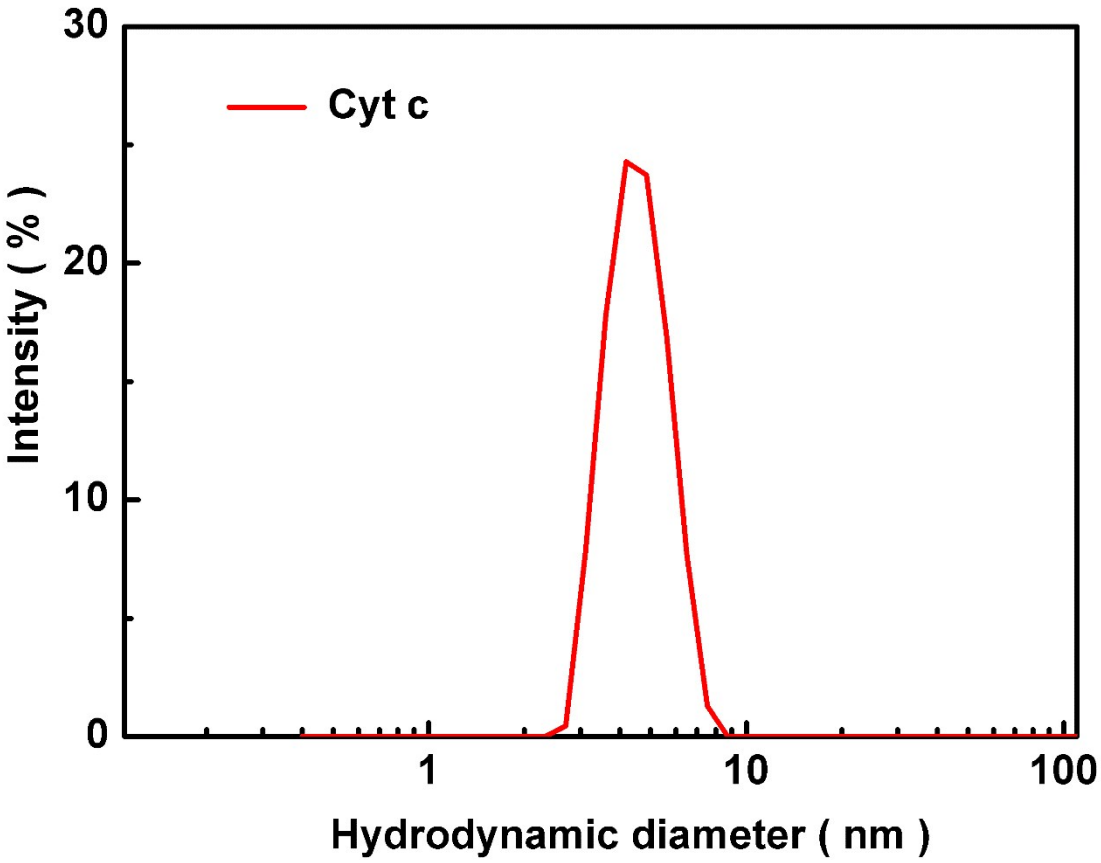


Fig. S5 Molecule size distribution of NBC modified Cyt c, measured by dynamic light scattering.

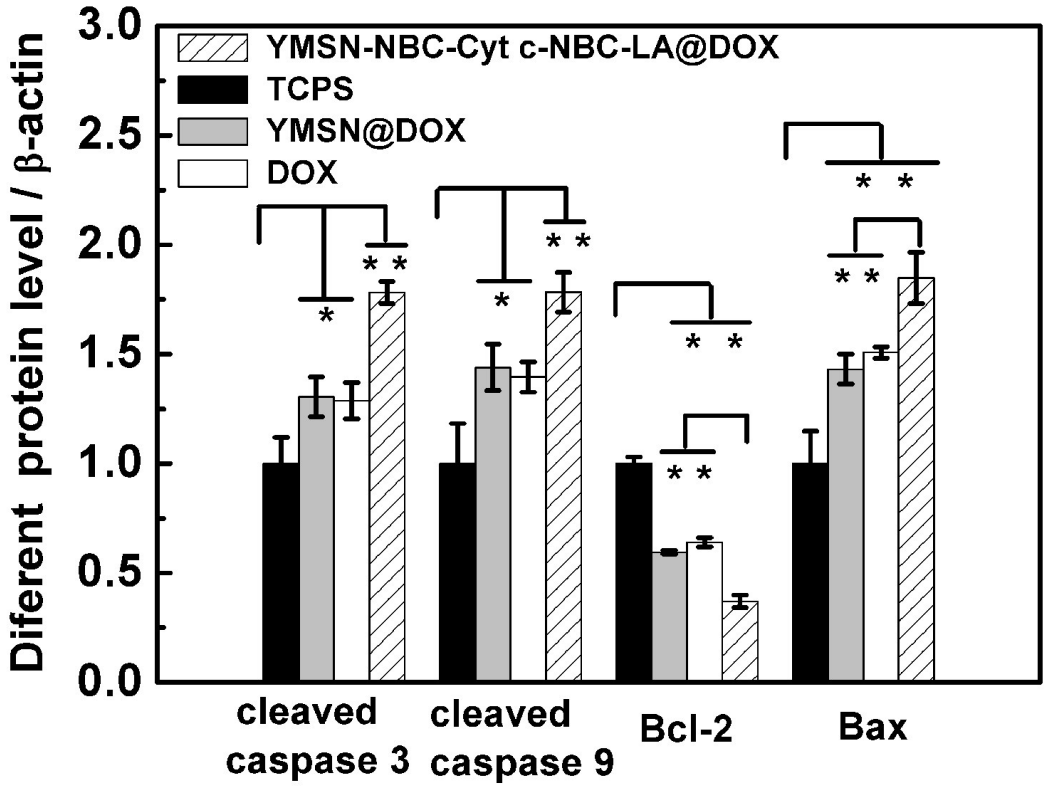


Fig. S6 Western blot analysis on the expression levels of cleaved caspase 3, cleaved caspase 9, Bcl-2 and Bax in HepG2 cells after incubation with TCPS, YMSN@DOX, DOX, and YMSN-NBC-Cyt c-NBC-LA@DOX.($p<0.05$,**$p<0.01$)

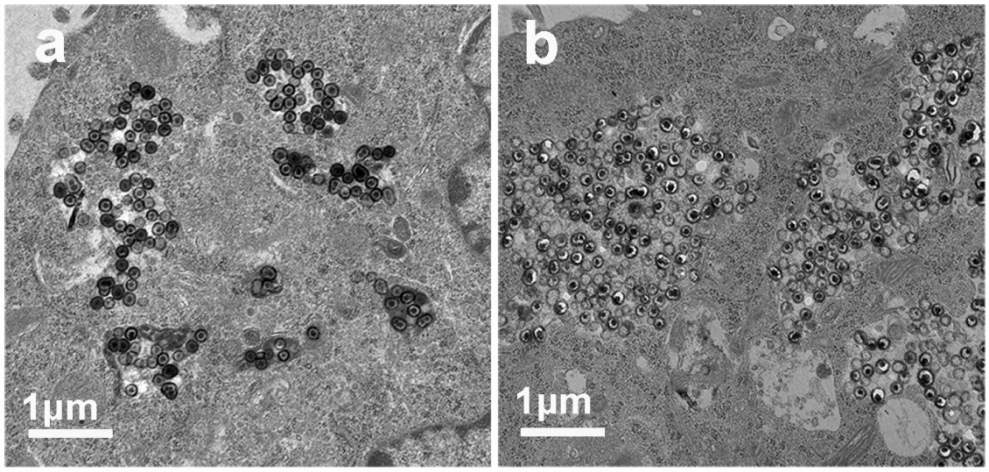


Fig. S7 Enlarged TEM images showing the endocytosed YMSN and YMSN-NBC-Cyt c-NBC-LA in HepG2 cells.

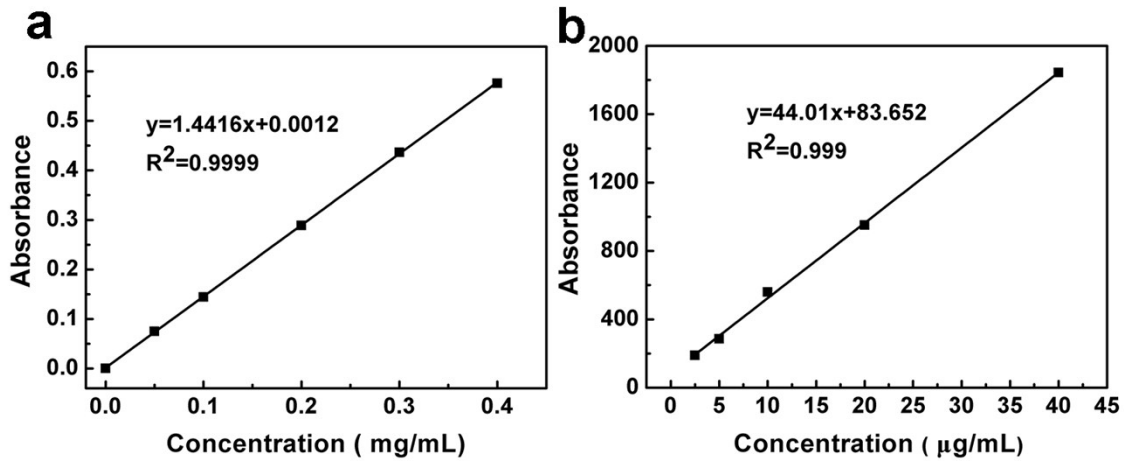


Fig. S8 Quantification of the DOX and Cyt c loading capacity. UV-VIS spectrophotometer was applied to investigate the DOX loading in the YMSN-NBC-Cyt c-NBC-LA@DOX. The DOX loading capacity (DLC) was calculated using the equation: DLC (%) = Amount of loaded drug / Total Weight of YMSN-NBC-Cyt c-NBC-LA@DOX ×100%. The Cyt c loading in the same nanospecies was measured on a fluorescence spectrophotometer, in which the Cyt c molecules were fluorescently labelled with FITC. The Cyt c loading capacity (CLC) was calculated using the equation: CLC (%) = Amount of loaded Cyt c / Total weight of YMSN-NBC-Cyt c-NBC-LA@DOX ×100%, Panel a is the concentration-dependengt standard curve of DOX and Panel b is the concentration-dependengt standard curve of Cyt c.

References.

1. M. Wang, S. Sun, C. I. Neufeld, B. Perez-Ramirez and Q. Xu, *Angew. Chem. Int. Ed. Engl.*, 2014, **53**, 13444-13448.
2. J. L. Major Jourden and S. M. Cohen, *Angew. Chem. Int. Ed. Engl*, 2010, **49**, 6795-6797.
3. Z. Luo, K. Cai, Y. Hu, J. Li, X. Ding, B. Zhang, D. Xu, W. Yang and P. Liu, *Adv. Mater.*, 2012, **24**, 431-435.