Supplementary Information

Interface active polydopamine for nanoparticles stabilized nanocapsules in a one-pot assembly strategy toward efficient drug delivery

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Characterizations

Transmission electron microscopy (TEM) images were obtained using a JEM 2010 (JEOL, Japan) instrument with 200 kV acceleration voltages to observe the size, morphology and integrity of the nanocapsules. Samples were dried on holey carbon-coated Cu grids. Hydrodynamic diameter distributions and zeta potentials were measured using a Zetasizer instrument (Malvern, UK). The ultraviolet absorptions were measured using an UV-Vis Spectrophotometer (NanoDrop One, Thermo) and fluorescence spectra were obtained using a fluorescence spectrophotometer (RF6000, Shimadzu). Fluorescence images were measured using a confocal laser scanning microscopy (TCS SP5, Leica). Flow cytometry analysis were obtained using a CytoFLEX instrument (BECKMAN COULTER). CCK-8 test was achieved using a

microplate reader (Multiskan Spectrum, Thermo Fisher, USA).

Determination of the drug loading capacity

To determine the drug loading capacity, an aqueous suspension of PTX loaded LA-Arg-PDA nanocapsules (1 mL) was mixed with absolute ethanol (1 mL), stirred for 1 h and separated by centrifugation to obtain the PTX containing supernatant. After diluted 10 times with water, the PTX solution was purified by using a high performance liquid chromatography (HPLC, LCMSMS-8060, Shimadzu, Japan) and the amount of PTX in the supernatant was measured at its characteristic absorbance wavelength, 227 nm. Chromatographic separation of PTX was carried out on a Shim-pack GISS C18 (2.1 × 50 mm 1.9 μm) analytical column with a mobile phase consisting of 65% methanol and 35% water, pumped at a flow rate of 1 mL min^{-1}. The retention time of PTX was about 1.2 min. For the determination of DOX loading capacity, the suspension LA-Arg-PDA nanocapsules after DOX adsorption was separated by centrifugal filtration (10000 rpm, 10 min, the cutting-off molecule weight: 3000) to obtain the supernatant. After diluted with sodium acetate buffer solution, the amount of DOX in the supernatant was measured by a fluorescence spectrophotometer at its excitation wavelength of 480 nm and emission wavelength of 585 nm. This measured fluorescence intensity was compared to a standard curve to determine the concentration of DOX. The amount of DOX loaded into LA-Arg-PDA nanocapsules was calculated by subtracting the mass of DOX in the supernatant from the total mass of drug in the initial solution.
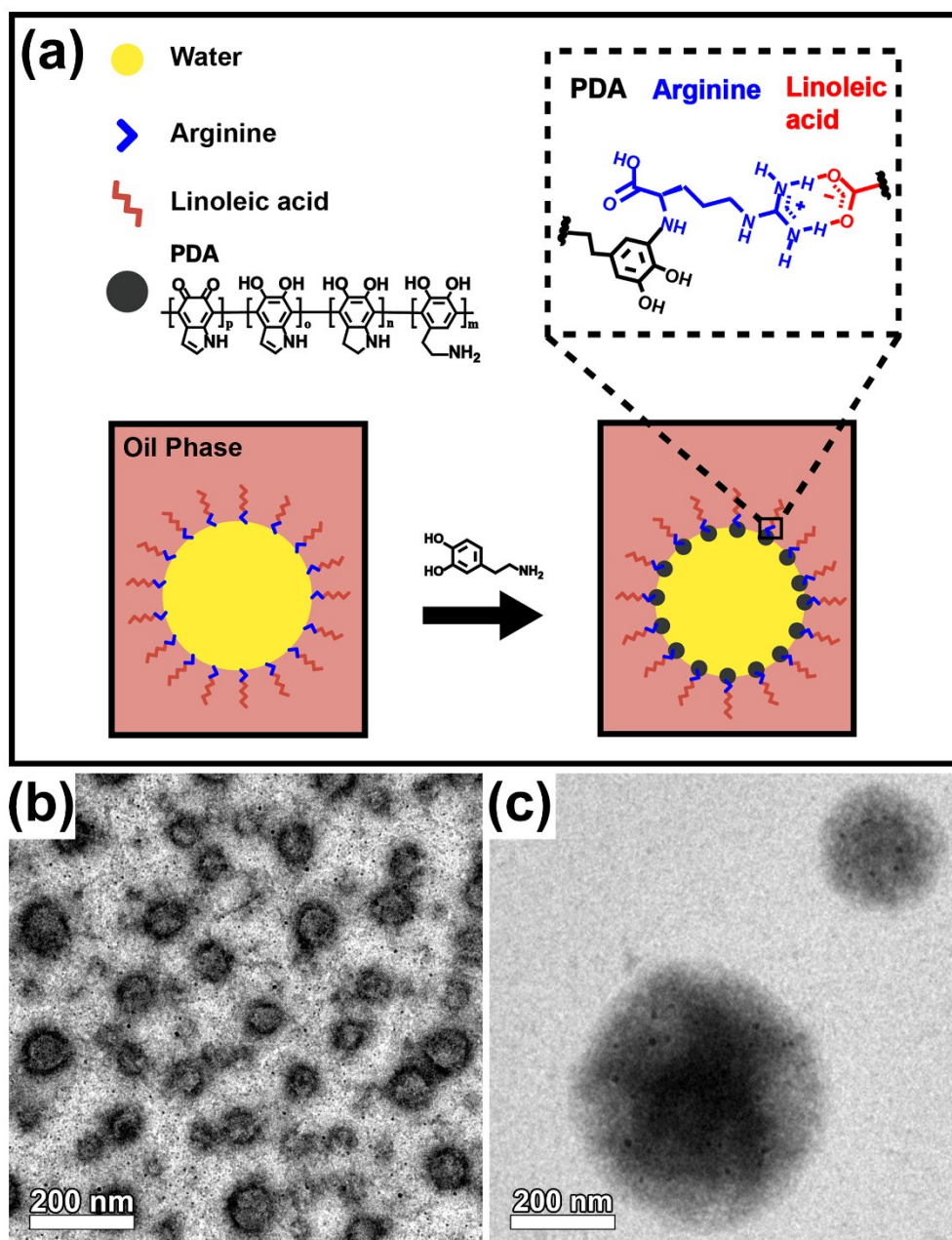
Supplementary figures.

Fig. S1 Schematic synthetic illustration (a) and TEM images of PDA-Arg-LA nanocapsules formed in a water-in-oil emulsion at varying dopamine loading: 60 mg (b), 30 mg (c). Image b and c were negatively stained using 2% phosphotungstic acid (pH=7) before characterization.
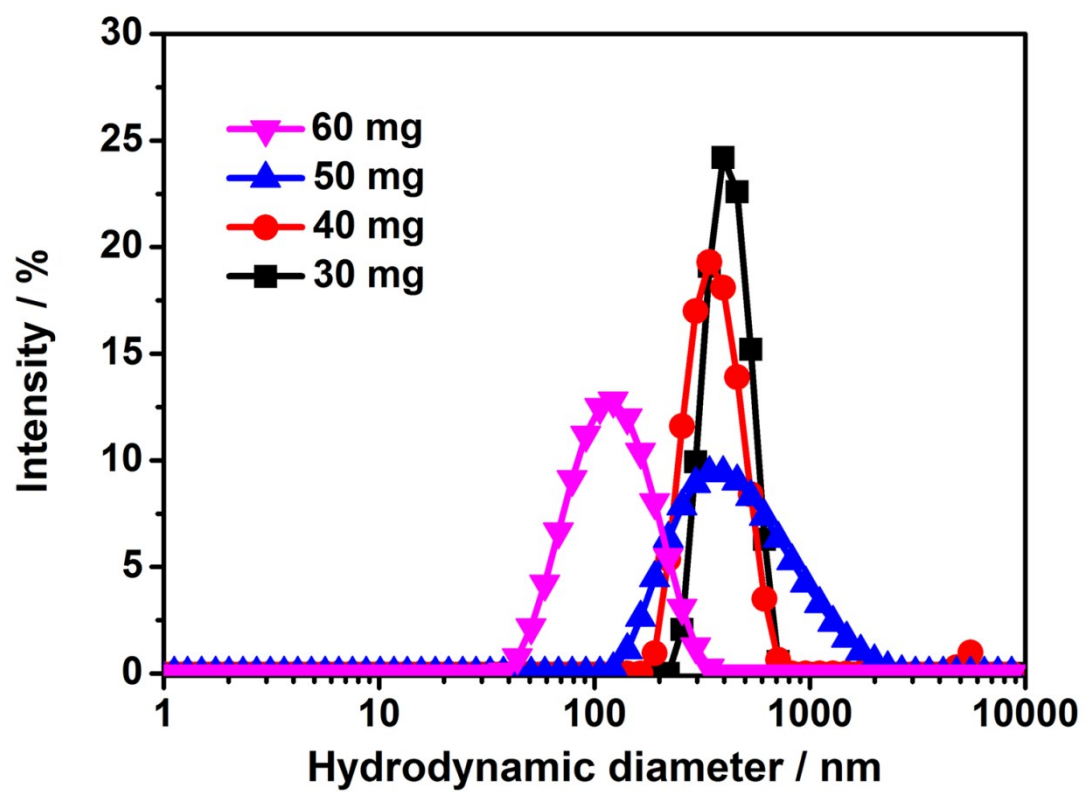
Fig. S2 Hydrodynamic diameter distributions of LA-Arg-PDA nanocapsules synthesized at varying amounts of dopamine.
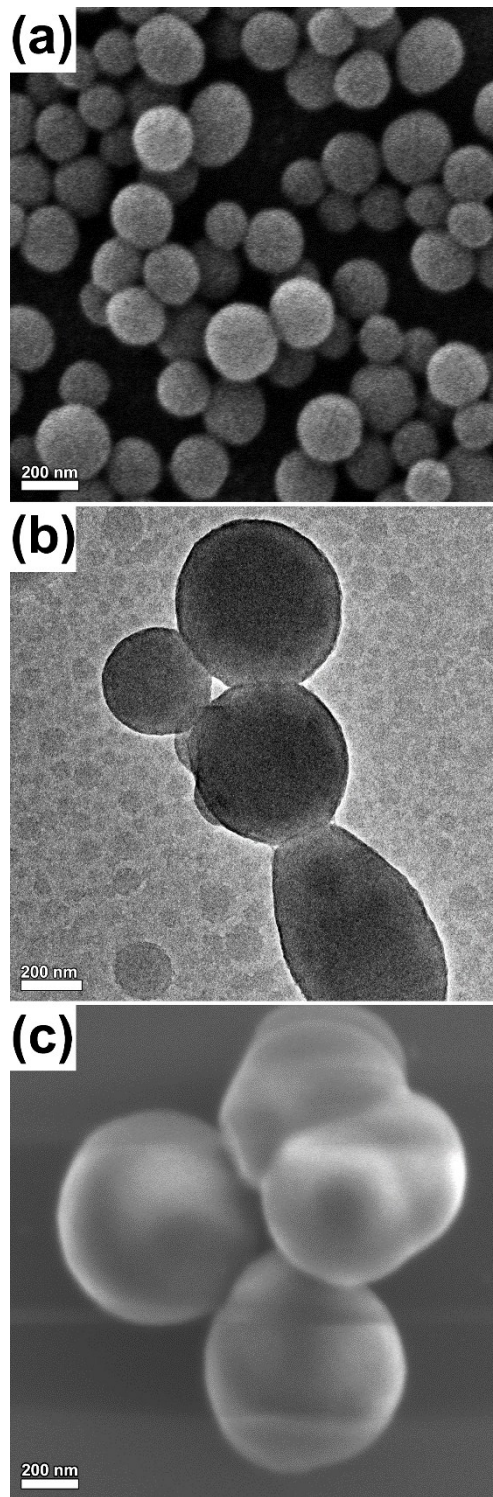
Fig. S3 SEM image of Arg-PDA nanoparticles (a), TEM image of LA-PDA nanocapsules (b), and SEM image of PDA nanoparticles (c).
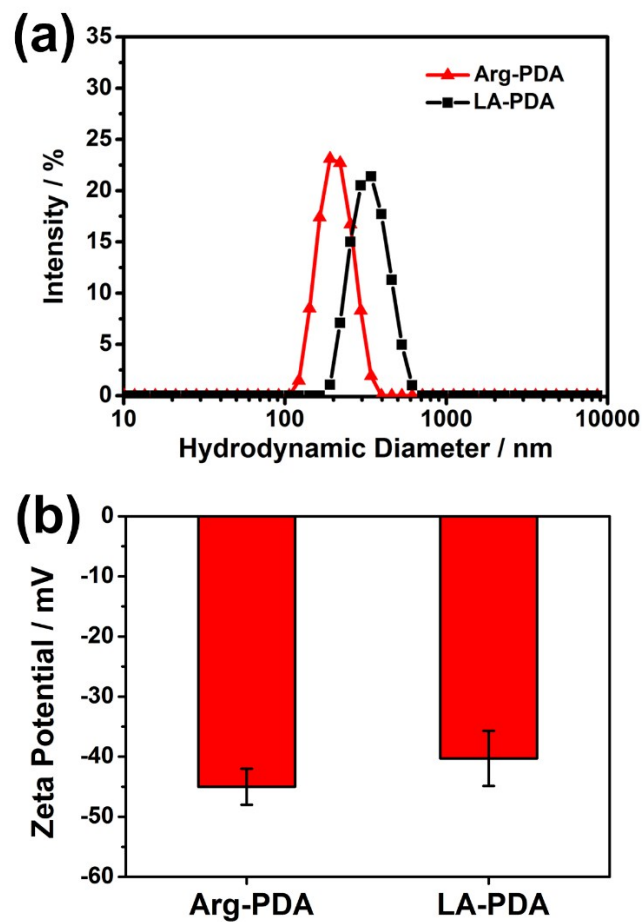
Fig. S4 Hydrodynamic diameter distributions (a) and corresponding zeta potentials (b)

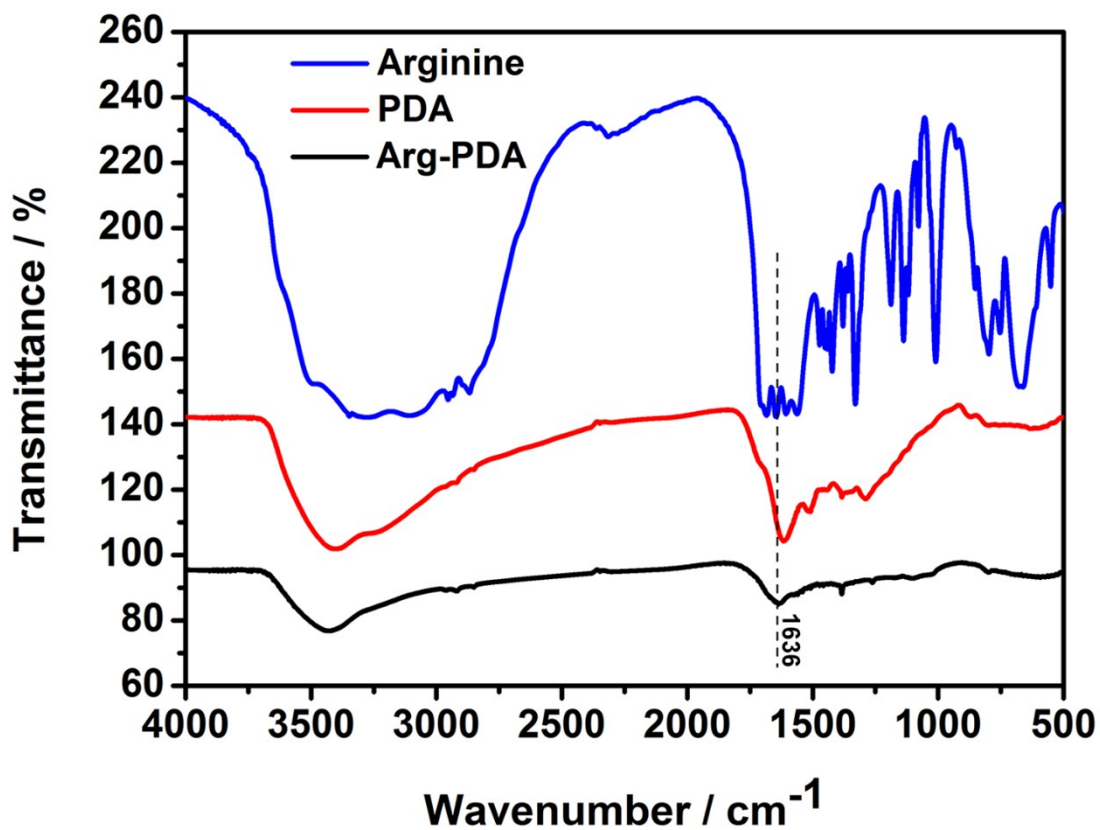of Arg-PDA nanoparticles and LA-PDA nanocapsules.

Fig. S5 FTIR spectra of arginine modified PDA nanoparticles and PDA nanoparticles. The new absorption band at 1636 cm^{-1} was assigned to the guanidine group,[1] indicating that there is no chemical reaction between guanidine group (pK_a=13)[2] and PDA.
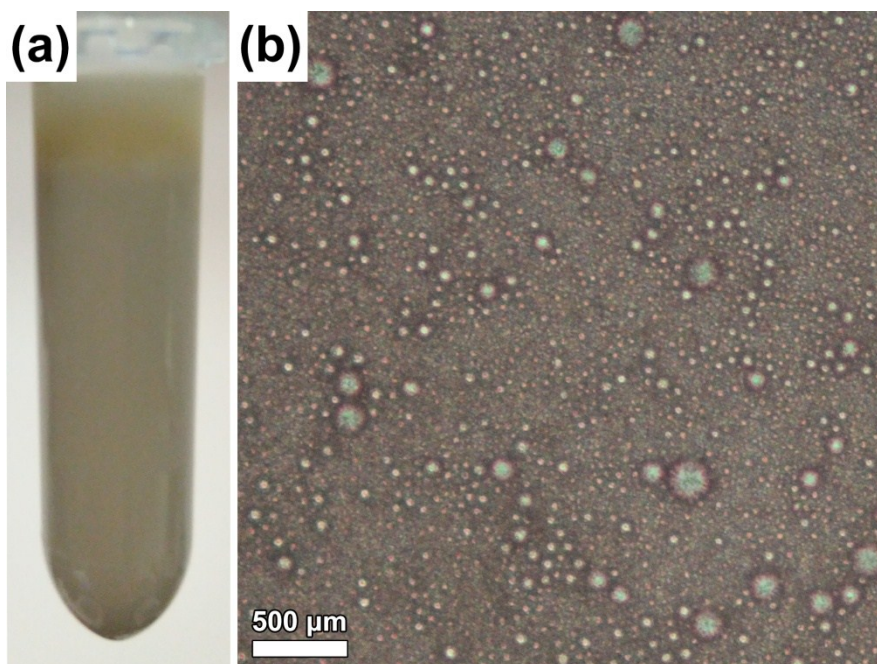
Fig. S6 (a) Digital images of aqueous suspensions of arginine modified PDA nanoparticles after mixing with linoleic acid (v/v, 90/10), followed by shaking for 10 s, ultrasonic vibrating for 20 min and keeping static for 12 h. (b) Optical microscope image of the lower water phase from mixture.
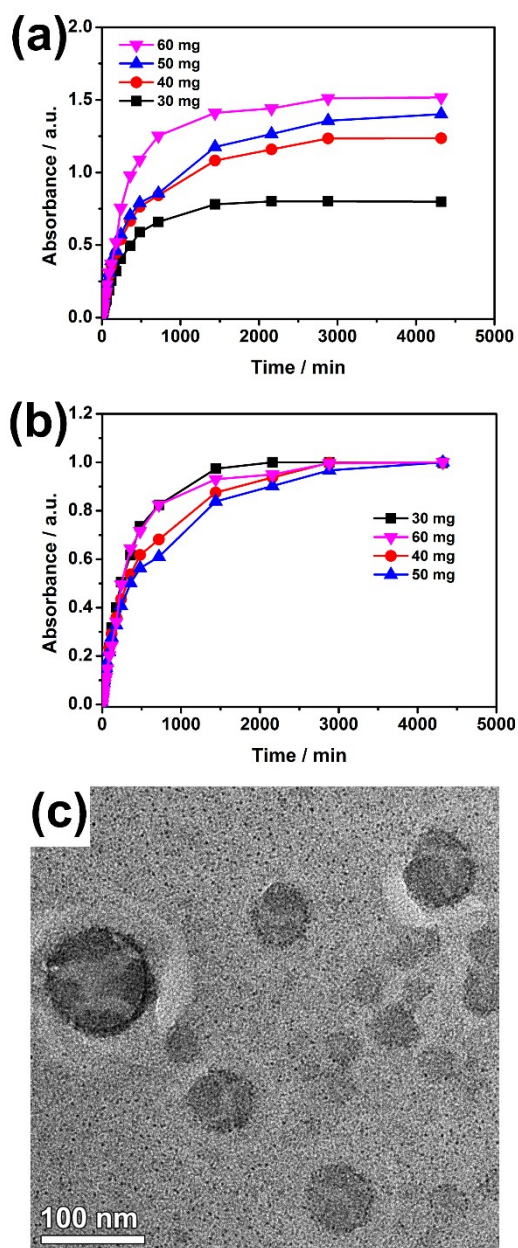
Fig. S7 Temporal evolution of the absorbance at 400 nm for LA-Arg-PDA nanocapsules synthesized at varying amounts of dopamine (a) and corresponding normalized curves (b). TEM image of the preparation solution of LA-Arg-PDA nanocapsules (c) in the intermediate stage of the reaction (24 h).
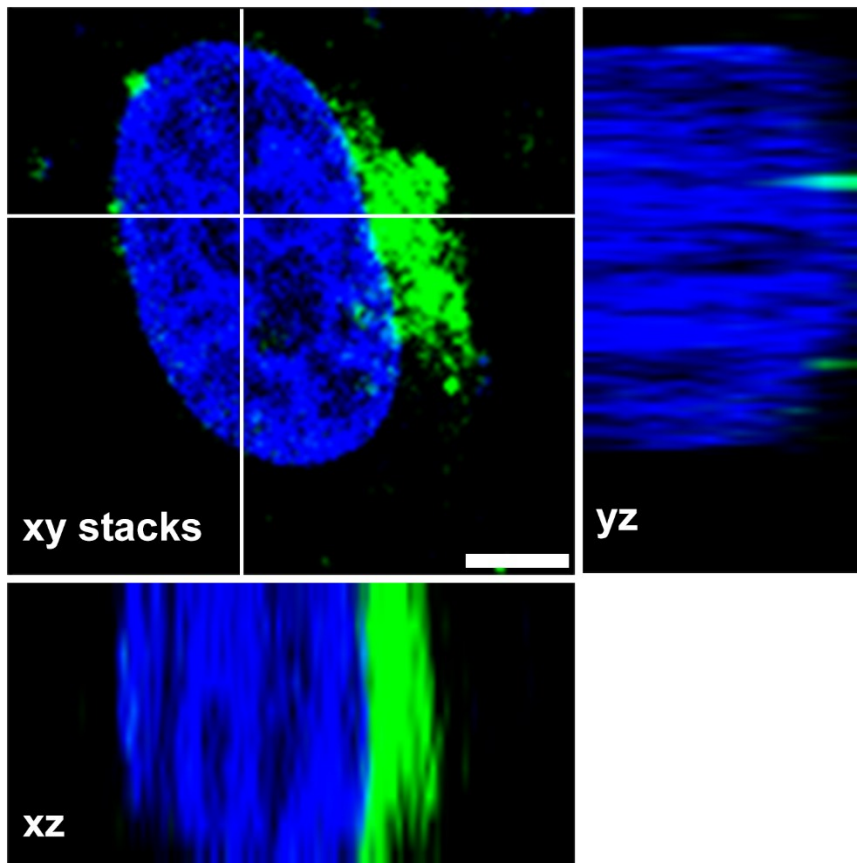
Fig. S8 Confocal laser scanning microscopy (CLSM) images of a Hep-G2 cell extracted from **Fig. 5c**. Large image is a compressed stack of confocal slices. White cross-lines delineate the plane of the individual slices shown to the right (vertical line) and below (horizontal line) each stack. Cell nuclei were stained with Hoechst 33258 (blue). Scale bar: 5 μm.
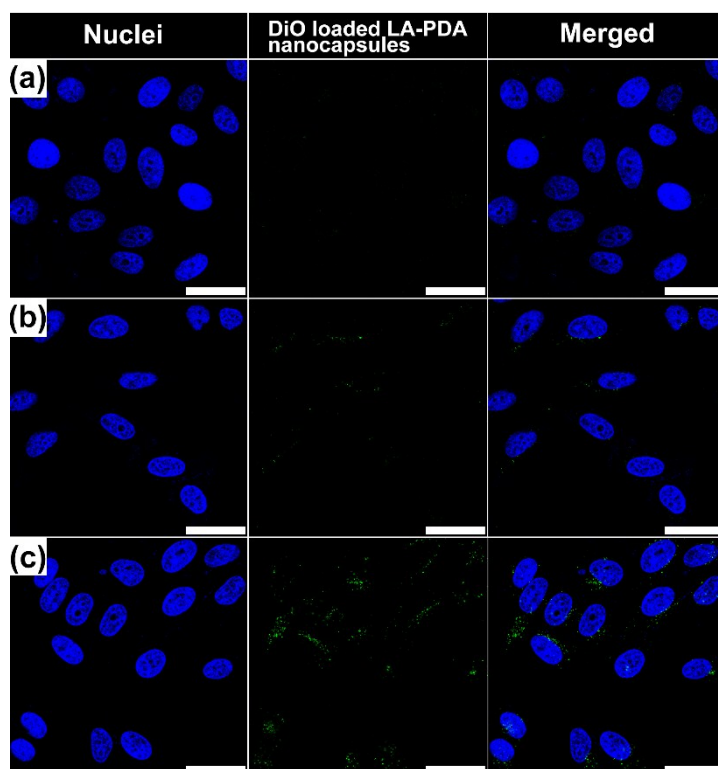
Fig. S9 Confocal laser scanning microscopy (CLSM) images of Hep-G2 cells incubated with DiO (green) loaded LA-PDA nanocapsules for 1 h (a), 3 h (b), and 6 h (c). Cell nuclei were stained with Hoechst 33258 (blue). Scale bar: 25 μm.
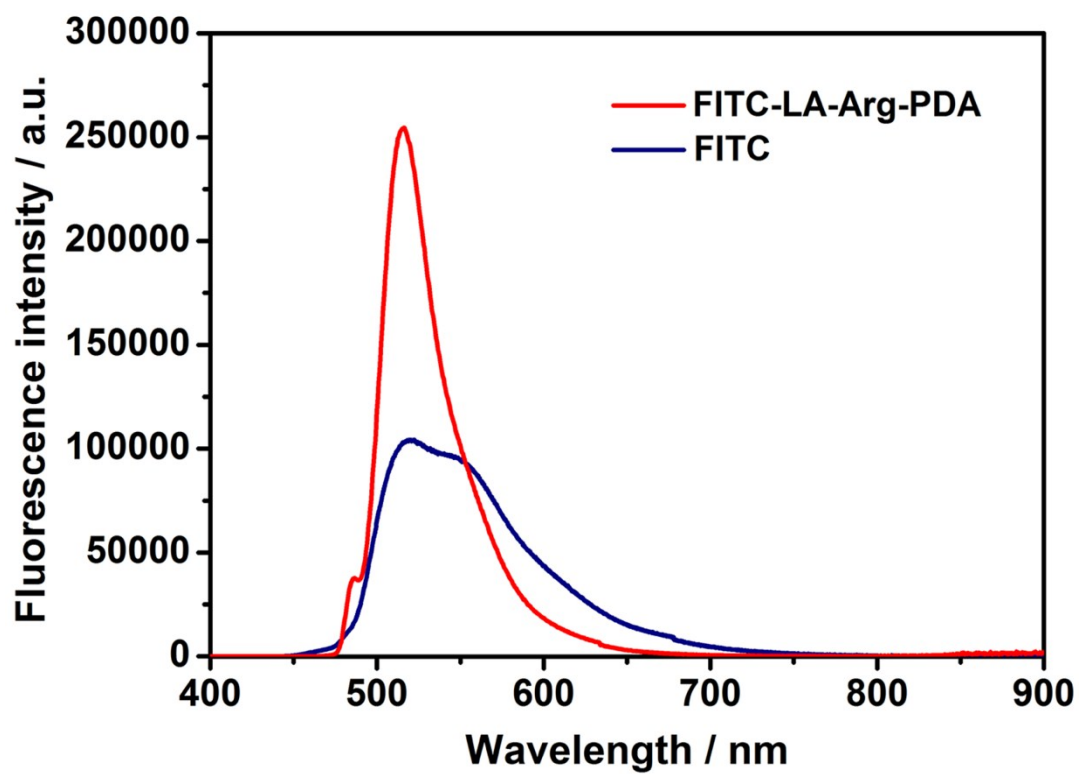
Fig. S10 Fluorescence emission spectra of LA-Arg-PDA nanocapsules labeled by SH-PEG-FITC and free FITC solution.
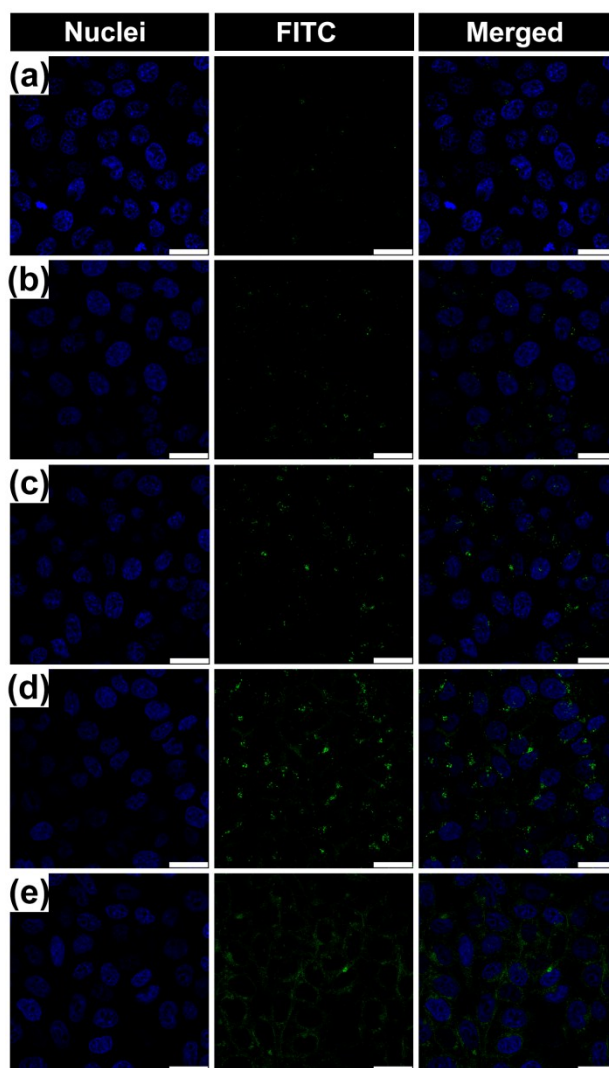
Fig. S11 Confocal laser scanning microscopy (CLSM) images showing the cell endocytosis of LA-Arg-PDA nanocapsules over incubation time: 1 h (a), 3 h (b), 6 h (c), 12 h (d) and 24 h (e). Cell nuclei were stained with Hoechst 33258 (blue), while nanocapsules were labeled with SH-PEG-FITC (green) by the Michael addition reaction between thiol groups and polydopamine. The scale bar represents 35 μm.
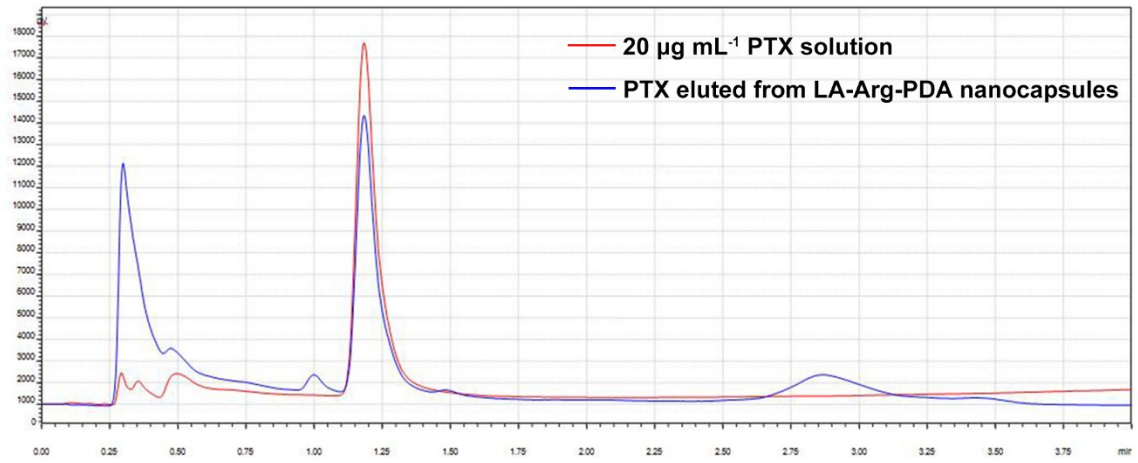
Fig. S12 HPLC spectra of a PTX solution (20 µg mL^{-1}) and PTX supernatant by ethanol elution, filtration centrifugation, and dilution, after PTX loading by LA-Arg-PDA nanocapsules.
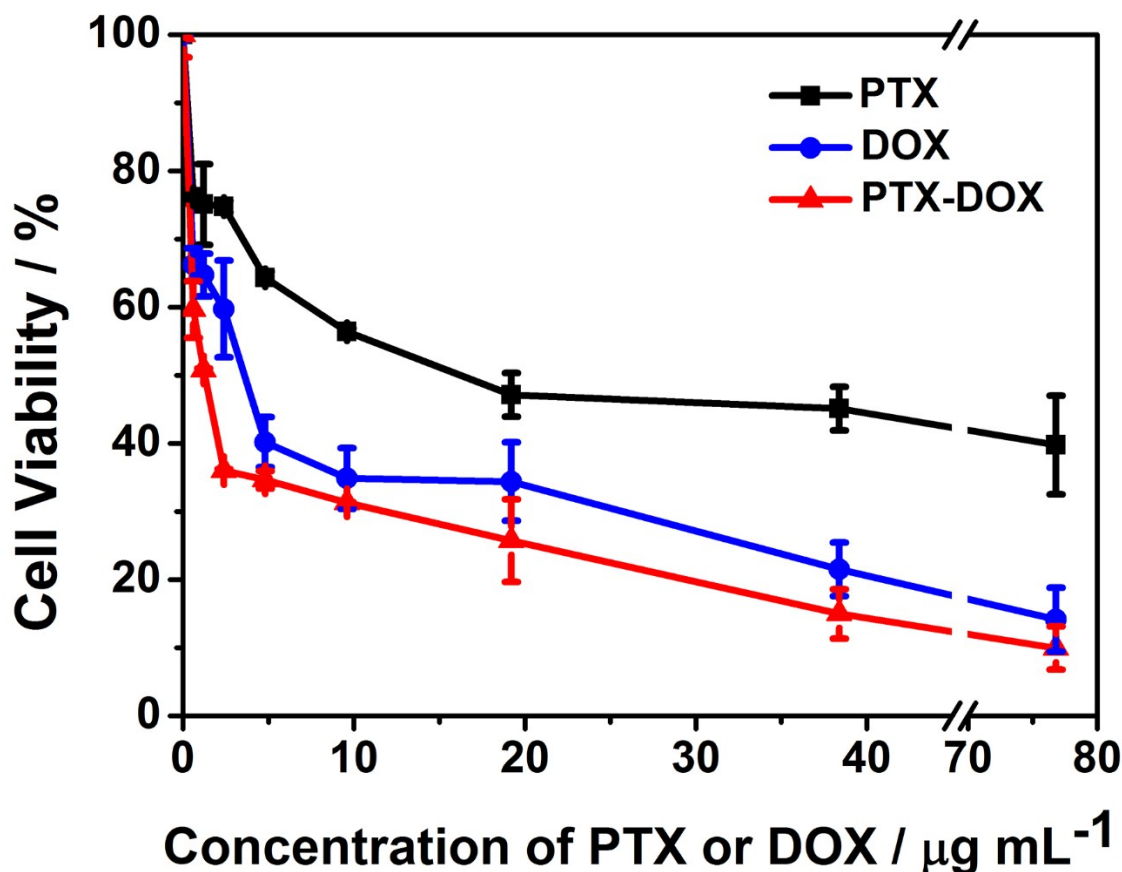
Fig. S13 Viability of Hep-G2 cells after incubated with varying concentrations of DOX (blue line), PTX (black line) or DOX-PTX combination (red line) for 24 h. The viabilities were determined by the CCK-8 test.

The degree of synergy was estimated by a combination index (CI) using the Chou-Talalay isobologram equation.[3, 4]

$$\frac{f_a}{f_u} = (\frac{D}{D_m})^m \tag{1}$$

In equation 1, D is the dose, D_m is the dose required for 50% effect (e.g., 50% inhibition of cell growth), f^a is the fraction affected by dose D (e.g., 0.9 if cell growth is inhibited by 90%), f_u is the unaffected fraction (therefore, $f_a = 1 - f_u$), and m is a coefficient of the sigmoidicity of the dose-effect curve; m = 1, m > 1，and m < 1

indicate hyperbolic, sigmoidal, and negative sigmoidal dose-effect curves, respectively.

A rearrangement of equation 1 is as follows:

$$D = D_m \left(\frac{f_a}{1 - f_a} \right)^{\frac{1}{m}}$$

(2)

If we obtained the single drug and two drug combination curve by CCK-8 test, the

D_m and m can be easily determined from the logarithmic form of equation (2). That is:

if x = log (D) versus y = log (f_a/f_u) then m is the slope and log (D_m) is the x-intercept.

To determine the combination index (CI) value, the combination index (CI)-

isobologram equation was used as follows.

$$CI = \frac{D_1}{(D_X)_1} + \frac{D_2}{(D_X)_2}$$

(3)

Equation 3 dictates that drug 1, i.e., (D)$_1$, and drug 2, i.e., (D)$_2$, in the numerators

in combination inhibit x%. (D$_x$)$_1$, and (D$_x$)$_2$ in the denominators of equation 3 are the

doses of drug 1 and drug 2 alone, respectively, that also inhibit x%. D$_x$ can be readily

calculated from equation 2, where D is designated for x% inhibition. CI<1, CI = 1, and

CI>1 indicate synergism, additive effect, and antagonism,

The Combination Index (CI) of the PTX (0.7 µM)-DOX (1.1 µM), PTX (1.4 µM)-

DOX (2.2 µM), PTX (2.8 µM)-DOX (4.4 µM), PTX (5.6 µM)-DOX (8.8 µM), PTX

(11.1 µM)-DOX (17.6 µM), PTX (22.4 µM)-DOX (35.2 µM), PTX (44.8 µM)-DOX

(70.0 µM), PTX (89.6 µM)-DOX (140.8 µM) combination was 0.51, 0.46, 0.25, 0.43,

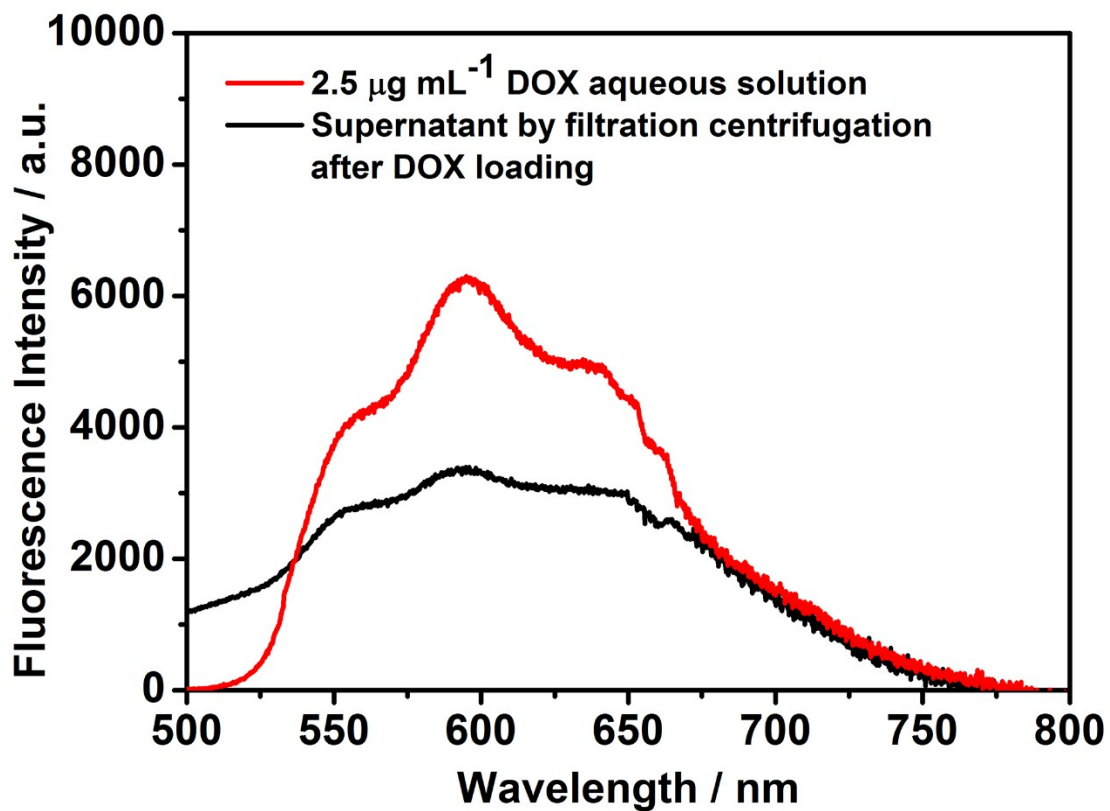0.62, 0.64, 0.32, 0.24, respectively, indicating a synergistic effect (CI<1).

Fig. S14 Fluorescence spectra of DOX solution (2.5 μg mL^{-1}) and the supernatant by filtration centrifugation (followed by dilution) after DOX loading by LA-Arg-PDA nanocapsules (initial DOX concentration: 0.3 mg mL^{-1}; nanocapsule concentration: 7.9 mg mL^{-1}).
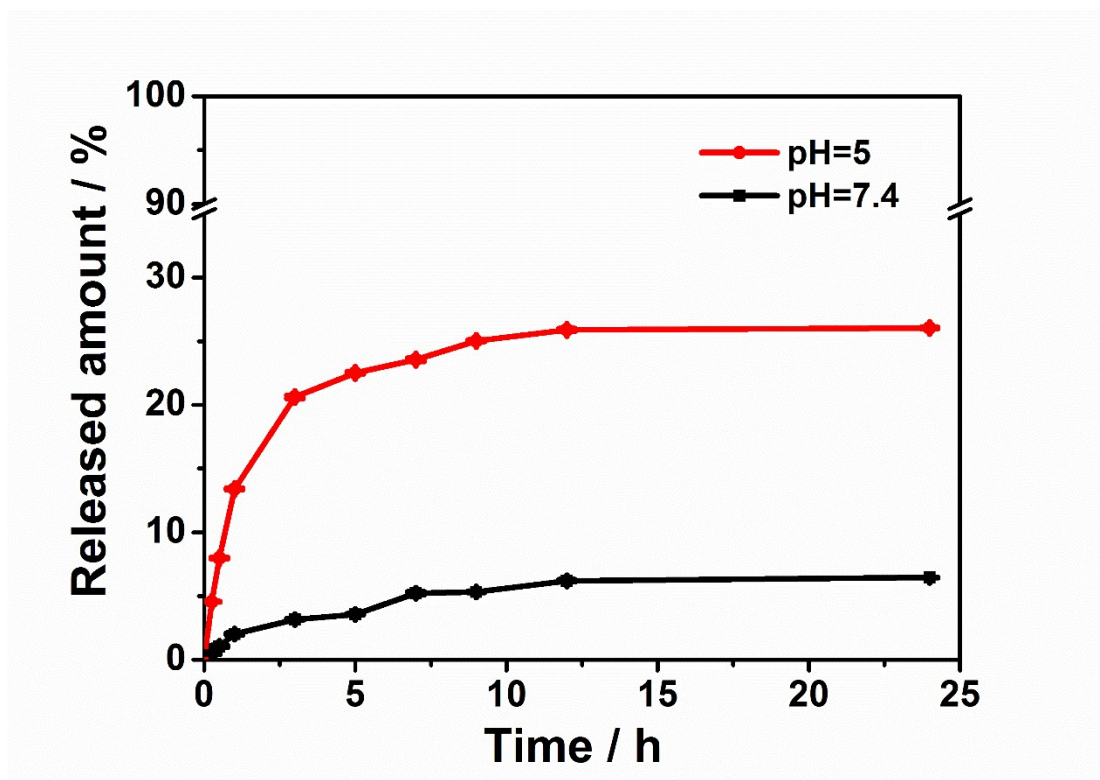
Fig. S15 Release profiles of DOX from the LA-Arg-PDA nanocapsules at two different
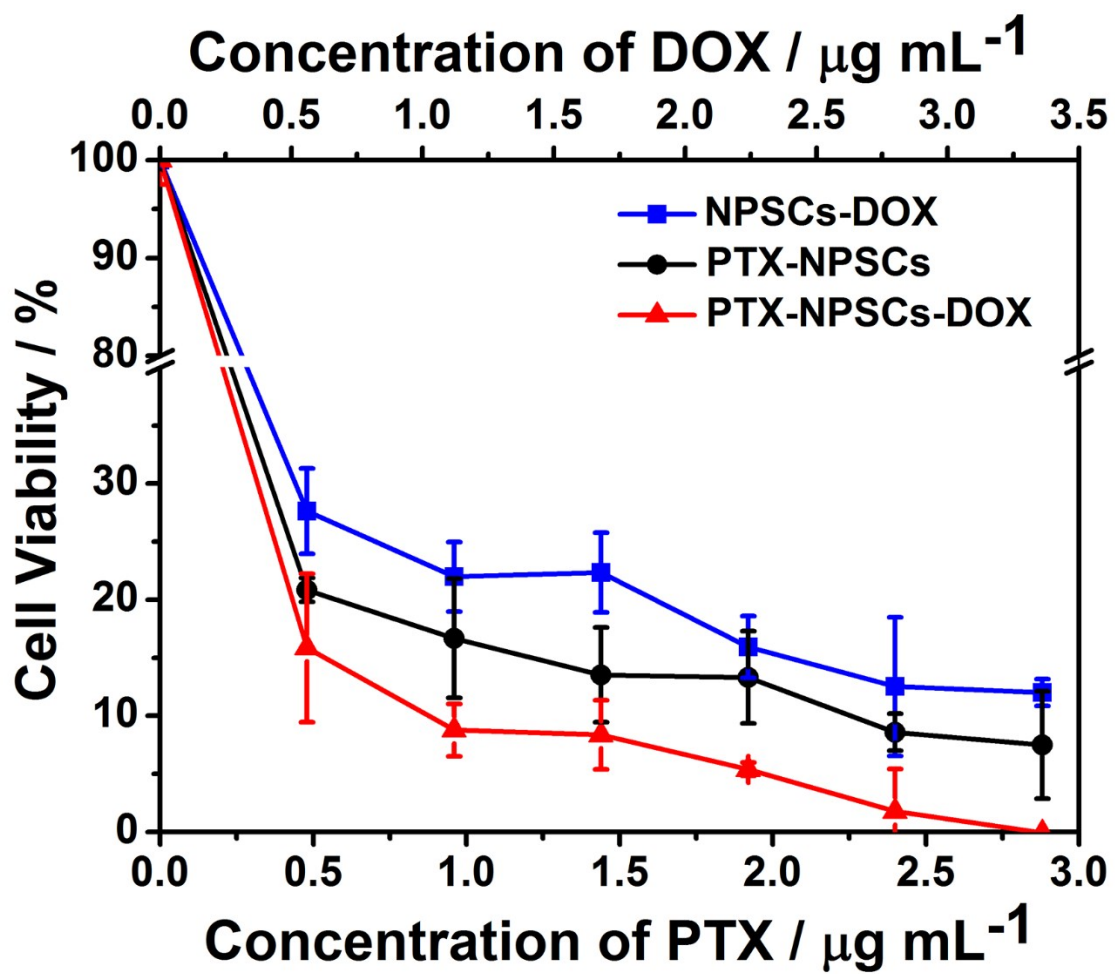
pH values (5 and 7.4).

Fig. S16 Viability of Hep-G2 cells after incubated with varying concentrations of DOX loaded (blue line), PTX loaded (black line) or DOX-PTX co-loaded (red line) LA-Arg-PDA nanocapsules for 48 h. The viabilities were determined by the CCK-8 test.

References

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2   J. Ji, W. Zhu, J. Li, P. Wang, Y. Liang, W. Zhang, X. Yin, B. Wu and G. Li *ACS Appl. Mater. Interfaces*, 2017, **9**, 19124-19134.

3   T.-C. Chou, R. J. Motzer, Y. Tong and G. J. Bosl *J. Natl. Cancer. Inst.*, 1994, **86**, 1517-1524.

4   Y. Zhang, Z. Hou, Y. Ge, K. Deng, B. Liu, X. Li, Q. Li, Z. Cheng, P. a. Ma, C. Li and J. Lin *ACS Appl. Mater. Interfaces*, 2015, **7**, 20696-20706.